ABSTRACT

SARAH CHILUNGO. Bioaccessibility of Beta Carotene in Processed Products from Orange-Fleshed Sweetpotatoes (Under the direction of Dr. Van Den Truong and Dr. Jonathan C. Allen, co-chairs).

Orange-Fleshed sweetpotatoes (OFSP) contain significant quantities of β-carotene, a precursor for vitamin A. The crop is being promoted to tackle vitamin A deficiency, a serious public health problem affecting children and pregnant/lactating women in sub-Saharan Africa. Significant studies have reported the efficacy of OFSP in improving serum retinol. The aim of the thesis was to evaluate the bioaccessibility of β-carotene in processed products from OFSP as affected by processing method and oil type. Bioaccessibility is the fraction or quantity of nutrients in food that is released from the matrix during digestion in the gastrointestinal tract and becomes available for absorption. Static *in vitro* digestion methods were used to determine carotene bioaccessibility. Bioconversion of β-carotene into VA and carotene retention stored in different packaging material were also investigated.

This first study evaluated the effect of storage and packaging materials on carotene content, color and water activity of OFSP flours. Flours from Vita and Kabode OFSP genotypes were packed in aluminum foil laminate (AFL), high density polyethylene (HDPE) and Kraft paper and stored under light and dark conditions for 4 months. The highest carotenoid loss after 4 months was found in Kraft paper while AFL was the least. Similarly, flours in Kraft paper registered significant color value changes compared to flours in HDPE and AFL. Significant increase in water activity was observed in all packed samples and results were not dependent of storage environment. It is concluded that AFL with vacuum sealing is an appropriate packaging material due to better carotene retention, minimal color change and less water activity.

Porridge and *chapati* were prepared with either puree or flour from the two sweetpotato genotypes. Digestive stability and bioaccessibility of the products were evaluated following *in vitro*

methods. Beta-carotene digestive stability of porridge was significantly lower than *chapatis*. In the same line, β-carotene bioaccessibility was significantly lower for porridge than *chapatis*. It was also found that puree-based products registered low β-carotene bioaccessibility compared to flour-based products. For all the products, the study found no effect of sweetpotato genotype of β-carotene bioaccessibility while similar products had comparable carotene bioaccessibility. The findings confirm the effect of processing methods on carotene bioaccessibilty.

Sunflower oil, margarine and beef fat were evaluated on their effect on β-carotene bioaccessibility. The amount of oil added was 10% (w/w) of *chapati* formulation. The results showed that digestive stability was non-significant among the products implying that oil type has no effect on digestive stability. Among the three oil types, sunflower oil had the highest carotene bioaccessibility followed by margarine while beef fat was the least. The low melting point of sunflower oil was the possible reason for high carotene bioaccessibility. Therefore, consumers should consider replacing margarine with sunflower oil due to low price and availability.

The study further investigated the *in vitro* bioconversion efficiency of β-carotene into VA to determine VA value of OFSP supplemented porridge and *chapati*. When β-carotene extract was incubated with chicken intestinal mucosa post mitochondrial fractions, retinal (RAL) and retinoic acid (RA) were formed. No RAL and RA were formed when pure all-trans β-carotene standard was incubated without chicken intestinal mucosa post mitochondrial fraction. On average, the conversion ratio of β-carotene to RAL was 5:1. The results are of significance to people in developing countries who depend on plant food to meet their VA requirements. Further investigation of the study is suggested to validate the findings.

Bioaccessibility of Beta Carotene in Processed Products from Orange-Fleshed Sweetpotatoes

by
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DEDICATION

To my family and academic advisors who made my study possible.

BIOGRAPHY

My name is Sarah Chilungo and I originate from Malawi. In 2015, I was awarded a BHEARD scholarship to study Ph.D. Food Science with North Carolina State University. Although my undergraduate degree was in Agricultural Extension, the attainment of MSc Food Science from Michigan State University was the beginning of my journey in the field of science. I have always been passionate about science and completion of Ph.D. Food Science from NCSU gives me all the confidence I need to undertake scientific research in the field of food science. I am very grateful to having learned from some of the best universities in the USA with remarkable professors.

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CHAPTER 1.

INTRODUCTION

Vitamin A is an essential micronutrient required for normal body growth and human health. Vitamin A deficiency, a condition emanating from inadequate intake of vitamin A in foods is a major public health problem worldwide. The problem is most prevalent in under-five children and pregnant/lactating mothers (FAO/WHO, 2002). Cases of vitamin A deficiency are very high in sub-Saharan Africa where about 33 million pre-school children are affected accounting to one third of global cases (West, 2002). Consequences of severe vitamin A deficiency include stunted growth, weak immunity, xeropthalmia and death (Sommer & West, 1996). Traditionally vitamin A supplementation is the main strategy to combat vitamin A deficiency whereby under-five children are given vitamin A capsules every six months. Food fortification is another approach involving addition of vitamin A in food products such as sugar, cereal, and oil to increase vitamin A intake among vulnerable groups. Recently, biofortification has been identified as a better sustainable strategy to provide various vitamins and minerals to the population through-out the year at low cost (Kósambo et al., 1998). The method works by enriching promising crop varieties with various vitamins and micronutrients to enhance their nutrient profile.

Orange-fleshed sweetpotato (OFSP) is one crop that has been biofortified with β-carotene, a precursor for VA. In Kenya, varieties with varying and significant amounts of provitamin A have been developed and released in the recent past (Ndolo et al., 2007). As a staple food in the larger sub-Saharan region, OFSP has an advantage over most fruits and vegetables in supplying the much needed provitamin A, thus helping to fight vitamin A deficiency. Compelling evidence is available of the potential contribution of OFSP to improve nutrition among the vulnerable groups. A South African based study proved that OFSP is effective in improving vitamin A status among school going children (Jaarsrveld et al., 2005). In

a separate study conducted in the rural setting of Mozambique, significant improvements in vitamin A intake and serum retinol concentration were obtained as a result of OFSP consumption (Low et al., 2007). The research-based evidence strengthens the importance of OFSP as a source of provitamin A and suggests the need to intensify research and advocacy to include the crop in diets of the vulnerable groups.

In Africa there are efforts through Vitamin A Partnership for Africa (VITAA) Initiative and also through a Gates Foundation Project led by the HarvestPlus Challenge Program (Reaching End Users) and Centre for International Potato (CIP) to promote the use of OFSP varieties. CIP, a part of a Consultative Group on International Agricultural Research in collaboration with various research institutes, plays a pivotal role in breeding and multiplying OFSP varieties. It also promotes processing of OFSP by working directly with the community as well processors to ensure increased consumption of OFSP based products and consequently eradicate vitamin A deficiency.

There is a dearth of scientific knowledge on the effect of processing on carotene bioaccessibility. The overall goal of the study was to understand the effect of OFSP traditional processing methods on β-carotene bioaccessibility and bioconversion into vitamin A. The study was necessitated by findings during preliminary visit to Malawi and Kenya, whereby it was observed that traditional OFSP products form an integral part of the food system and source of income among rural communities. The work reported in this thesis provides some insights on processing methods with optimal carotene delivery and also the conversion rate of β-carotene into vitamin A after digestion. The information should help plant breeders to develop OFSP genotypes rich in carotene content. The information is also important to food processors as it will

guide them to processing methods with high carotene retention and delivery after digestion. In general, the information will help to tackle vitamin A deficiency in Africa.

1.1. Hypothesis and research objectives

The main hypothesis of the research work was that various OFSP genotypes could be processed into products with high carotenoid content and *in vitro* bioaccessibility. The specific objectives of the study were:

1. To study the effect of storage on sweetpotato flour color and carotenoid content as affected by packaging material
2. To identify and quantify carotene in traditional OFSP products as affected by sweetpotato genotype and processing
3. A. To assess the effect of sweetpotato genotype and processing technologies on the bioaccessibility of carotene through an *in vitro* digestibility method

   B. To determine bioconversion efficiency of beta-carotene into Vitamin A in OFSP products through *in vitro* methods
4. To study the effect of oil type used in cooked sweetpotato products on carotene retention and *in vitro* bioaccessibility of OFSP products.

REFERENCES

FAO/WHO Rome. (2002). Vitamin A in Human Vitamin and Mineral Requirements. Report of joint FAO/WHO expert consultation Bangkok, Thailand

Jaarsveld, P. J. V., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., and Benade, A.J. (2005). Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. American Journal of Clinical Nutrition, 81: 1080-1087

Kósambo, L., Carey E. E., Misra, A. K., Wilkes J., and Hagenimana, V. (1998). Influence of age, farming site, and boiling on pro-vitamin A content in sweet potato (*Ipomoea batatas* (L.) Lam.). Journal of Food Analysis, 11: 305-321

Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F., and Tschirley, D. (2007). A food based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. Journal of Nutrition, 137: 1320-1327

Ndolo, P. J., Nungo, R. A., Kapinga, R. E., and Agili, S. (2007). Development and promotion of orange-fleshed sweetpotato varieties in Western Kenya. Proceedings of the 13th ISTRC Symposium, 689 – 695

Sommer, A., and West, K. P. (1996). Vitamin A deficiency: Health, survival and vision. New York, Oxford University Press

West, K. P. (2002). Extent of vitamin A deficiency among preschool children and women of reproductive age. Journal of Nutrition (132): 2857-2866

CHAPTER 2.

LITERATURE REVIEW

2.1. Vitamin A Deficiency in Africa

Vitamin A (retinol) is an essential nutrient required in small amounts for normal functioning of the visual system, growth and development, and maintenance of epithelial cellular integrity, immune function, and reproduction by humans (WHO/FAO, 2004). In the diet, vitamin A is found in two forms, namely preformed and provitamin A. In animal foods, preformed vitamin A occurs as retinyl esters of fatty acids in association with membrane-bound cellular lipid and fat-containing storage cells. Provitamin A carotenoids in plant foods are also associated with cellular lipids but are embedded in complex cellular structures such as the cellulose-containing matrix of chloroplasts or the pigment-containing portion of chromoplasts (WHO/FAO, 2004). Green leafy vegetables (spinach and amaranth), yellow vegetables (pumpkins, squash and carrots) and yellow and orange non-citrus fruits (mangoes, papayas, and apricots) are good sources of provitamin A (Rodriguez Amaya, 1996). Preformed vitamin A is more bioavailable than provitamin A. This becomes a problem among low income people especially in developing countries who depend on plant foods as source of vitamin A, making them more susceptible to vitamin A deficiency.

Vitamin A deficiency is a condition that results from insufficient intake of vitamin A in the diet causing low concentration of the nutrient in body tissue leading to adverse health consequences even if there is no evidence of clinical xerophthalmia (WHO, 1996). The main specific sign and symptom of vitamin A deficiency is xerophthalmia and risk of irreversible blindness. Other nonspecific symptoms include; increased morbidity and mortality, poor reproductive health, increased risk of anemia, and contributions to stunted growth and development. The populations at risk are children, pregnant/lactating mothers and immune compromised individuals such as HIV positive people (Sommer 1998). Global vitamin A

deficiency prevalence is categorized according to seriousness on the basis of clinical and subclinical indicators of deficiency as shown in Table 1-1.

Daily vitamin A requirements for different groups were evaluated and intake levels were developed by FAO/WHO in order to tackle vitamin A deficiency (Table 2-2). The mean requirement for an individual is defined as the minimum daily intake of vitamin A, expressed as mg retinol equivalents (mg RE), to prevent xerophthalmia in the absence of clinical or subclinical infection. The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A-dependent functions and to maintain an acceptable total body reserve of the vitamin (WHO/FAO, 2004). The values were set with consideration for bioavailability of preformed vitamin A and provitamin A diets with adequate fat.

2.2. Importance of sweetpotato

Sweetpotato (*Ipomea batata* (L.) Lam.) is a dicotyledonous plant belonging to the *Convolvulacae* family. In Sub-Saharan African, sweetpotato is an important food crop. Sweetpotato plays an important role as a food security crop, providing most of the dietary carbohydrate in sub-Saharan Africa particularly during drought due to its ability to tolerate drought (Hagenimana & Owori, 1996). The high yielding characteristic of sweetpotato is an important attribute both as a food security crop and source of income among low income communities (Woolfe, 1992). The short growing period (3 - 4 months) compared to most crops strengthens the importance of sweetpotato as source of food (Woolfe, 1992). Sweetpotato can produce significant yield in infertile soils, something which is not possible with crops such as maize. High nutrient level like energy, fiber, minerals and vitamins have tremendously increased

the popularity of sweetpotato. Depending on flesh color, the types of sweetpotato include white, cream, yellow, purple and orange fleshed. Orange-fleshed sweetpotatoes (OFSP) are particularly important because of high β-carotene content, a provitamin A nutrient, hence a better and sustainable alternative to other flesh colors for vitamin A deficiency mitigation. Despite all these advantages of sweetpotato, the potential of the crop is not well exploited. In most societies sweetpotato is considered as a poor man's crop. Sweetpotato only becomes important as a relief crop when drought strikes after other crops like maize and rice have failed.

2.3. Sweetpotato production trends and consumption in Africa

Over the years there has been a tremendous increase in hectarage and production of sweetpotato in Africa. The volume of sweetpotato production almost tripled from 6 million tons in 1993 to 18 million tons in 2013 (Figure 2-1) (FOASTAT). Although consumption of white-fleshed sweetpotato still dominates, introduction and adoption of OFSP has increased the area that grows sweetpotato as well as total crop production. Awareness campaigns and nutrition education by research institutes and partners have increased demand for new varieties, planting materials and knowledge to diversify utilization. Since OFSP is mainly used for domestic consumption, increased production has led to increased consumption by both urban and rural consumers in a number of countries. Significant increase in OFSP consumption has been reported in some African countries. In Nigeria, the sweetpotato consumption increased from 4 to 39 grams per person per day between 1992 and 2011, while in Mali it increased from 4 to 51 grams per person per day in the same period (source FAOSTAT and CIP). In a South African study, the OFSP was well received and even popular among children, who particularly liked its taste. About two thirds of the study group stated they would eat more than the serving provided

at school (Jaarsveld et al., 2005). The increased OFSP consumption offers hope to eradicate vitamin A deficiency in Africa.

2.4. Uses of sweetpotato

In sub-Saharan Africa, traditional methods of sweetpotato utilization include boiling, roasting and drying (Hall et al., 1998). Boiled sweetpotatoes are taken as part of breakfast, snack or as main meal during lunch or dinner (Woolfe, 1992). Recently, dried sweetpotato chips and flour have become important traditional products as a source of income for local producers. Dried chips are milled into flour and used to make porridge, doughnuts, and flat bread (*chapati*) among others (Nungo et al., 2000; Woolfe, 1992). The advent of OFSP has increased the importance of sweetpotato both at household and commercial level. Food companies have taken keen interest to use OFSP as raw material in various products to increase vitamin A intake and help fight vitamin A deficiency. For instance, in Malawi. Universal industries is producing crisps made from OFSP. In Kenya, OFSP bread and flour are available on the market while companies in Rwanda produce biscuit and juice (Figure 2-2). As observed during a preliminary research visit to Malawi and Kenya, the OFSP industry is still in the infant stage, hence the need for scientific research on OFSP processing to support the industry.

2.5. PROCESSING TECHNOLOGIES, CAROTENE BIOACCESSIBILITY AND METABOLISM

2.5.1. Carotenoids structure and properties

Carotenoids are C40 tetraterpenoids built from eight C5 isoprenoid units in an extensive conjugated double bond system, which serves as the light-absorbing chromophore responsible

for the yellow, orange, or red color in fruits and vegetables (De Moura, 2015). They are found in fruits and vegetables as carotenes (unsaturated hydrocarbons) and xanthophylls (oxygenated derivatives). Over 700 carotenoids have been identified in nature, the most prevalent carotenoid being β-carotene, α-carotene, lutein, lycopene, β-cryptoxanthin and zeaxanthin (Maiani et al., 2009). Presence of at least one β-ionine ring residue is required for a carotenoid to effectively function as provitamin A. β-carotene, α-carotene and β-cryptoxanthin are the three most commonly found provitamin A carotenoids in food consumed by humans (Figure 2-3). Only β-carotene has two β-ionone rings in its structure and is the most widespread of all carotenoids in food. Structurally, β-carotene can generate two molecules of vitamin A (retinol) when centrally cleaved or hydrolyzed, whereas α-carotene and β-cryptoxanthin can generate only one molecule (De Moura, 2015; Maiani et al., 2009). This makes β-carotene the most important carotene hence an important component of diet.

The absence of polar groups in their structure make carotenes practically insoluble in water. Consequently, the carotenes often form stable crystals due to their elongated, symmetric chain structure (Craft et al., 1992; Britton 1995). To a certain extent, the crystalline form significantly increases the stability of carotenes. However, carotenes are more susceptible to degradation and isomerization, especially in the presence of oxygen or heat (Delgado-Vargas et al., 2000). During isomerization, the all-trans β-carotene changes to cis-β carotene, mostly 9-cis, 13-cis, and 15-cis with low provitamin A activity. Autoxidation occurs when the double bonds of carotenes interact with oxygen or light and it renders them ineffective as provitamin A nutrients. It is therefore, important to minimize autoxidation of carotenes when handling OFSP in order to fully benefit from the nutrient.

The presence of large number of double bonds in carotene structure imply that a great number of geometrical isomers are theoretically possible. For instance, β-carotene the most abundant carotene in OFSP has 272 possible isomers. However, due to steric hindrance, only a few of these will be encountered in nature (Olson et al., 1995). The bent structure of carotene cis isomers renders them weak and bulkier and consequently their function is completely different from their all-trans counterparts. Furthermore, the weak structure of cis-isomers makes them less thermodynamically stable, hence not readily integrated into cellular structure. According to Britton, (1995) the bulkier structure of the cis-isomers also makes them less prone to aggregate or form crystals, which has a further negative impact on their stability during thermal processing.

2.5.2. The role of carotene in human health

Over the years carotenoids importance has increased due to their ability to prevent various diseases such as in eye, on heart or various types of cancer. Among the carotenoids, both α and β carotene have pro-vitamin A activity hence metabolize into vitamin A in the body. This is the basis of their improvement of human health as they perform vitamin A functions in maintaining health eye. Although epidemiological studies cannot provide cause and effect relationship, they have provided sufficient evidence about the health benefits of carotenoids. Scientific evidence shows that high consumption of fruits and vegetables rich in carotenoids reduced risk of lung and stomach cancers (Block et al., 1992). According to Block et al. (1990) the mechanism through which carotenoids prevent cancer as provitamin A is controlling cell differentiation and proliferation. The antioxidant potential of carotenoid is also mentioned as the mechanism of cancer prevention. The presence of conjugated double bonds makes carotenes effective antioxidants. This has been confirmed *in vitro*; for instance, carotenes significantly

reduced the oxidative response of lymphocytes and low density lipoproteins (LDL) isolated from humans (Martin et al., 1996; Oshima et al., 1996; Porrini et al., 2005). Although there is sufficient evidence on the antioxidant activity of carotene *in vitro*, the antioxidant *in vivo* activity is not clear. On the other hand, there is some contradictory information whether carotenes can function as pro-oxidants in high concentration (El-Agamey et al., 2004). This remains a potential researchable area to get insights on antioxidant and pro-oxidation properties of carotenes and their potential for disease prevention.

2.5.3. Defining bioaccessibility

Bioaccessibility is defined as the fraction or quantity of nutrients in food that is released from food matrix during digestion in the gastrointestinal tract (GI) and becomes available for absorption (Heaney, 2001). The process includes digestive transformation of food into material ready for absorption/assimilation into intestinal epithelia. In contrast, digestibility specifically refers to the fraction of food that is broken down by digestive enzymes and is potentially available for all physical and chemical reactions in the lumen, while absorption or assimilation is uptake of bioaccessible material through the epithelium by some mechanism of transepithelial absorption (Heaney, 2001).

In the lab set-up, bioaccessibility is usually evaluated through *in vitro* digestion methods mimicking oral, gastric and intestinal digestion. This is sometimes followed by cell culture using Caco-2 cells to assess nutrient uptake (Courraud et al., 2013). Caco-2 cells' absorption is preceded by *in vitro* digestion to measure the available carotenoid released from food matrix. Subsequently, the digested material is filtered out and added to Caco-2 cells and incubated for absorption, after which filtrate from the aqueous portion is collected to estimate absorbed

carotenoids. Main steps in carotenoid absorption include release from the food matrix, micelle formation, uptake into mucosal cells, packing into chylomicrons, and transport within the lymphatic system. Several studies have demonstrated that carotenoid bioaccessibility is affected by several factors, both endogenous (product related) and exogenous (process related) (Garret et al., 1999; Hendren 2002).

2.5.3.1 *In vitro* method of estimating bioaccessibility

Due to the limitations and high cost of *in vivo* models to estimate bioaccessibility, *in vitro* models have been developed as a more simple, inexpensive and reproducible alternative. The models are applied to study nutrient digestibility, release from the food matrix, micellarization, and intestinal uptake, and consequently to estimate their bioaccessibility. Two *in vitro* methods namely dynamic and static have been developed. The dynamic models such as the gastro-intestinal model (GIM) is characterized by a high degree of complexity closely modeling the GI system (Minekus et al., 1999).

In the current study, the static *in vitro* method was followed. The *in vitro* method involves use of a number of enzymes, pH adjustments and incubation at 37°C, simulating stomach and intestinal digestion. The oral phase of *in vitro* digestibility model involves incubating sample with alpha amylase enzyme at salivary pH. During the gastric phase, samples are incubated at gastric pH in the presence of gastric enzymes such as pepsin. During the small intestinal phase, pH is increased followed by addition of pancreatic enzymes and bile salts. In some studies, the oral phase is included to break down starch as some nutrients might be trapped inside starch molecule (Garret et al., 1999; Bengtsson et al., 2009). The Caco-2 cell model has extensively been used as a measure of bioavailable carotene after simulated enzymatic digestion.

2.5.3.2. Caco-2 cell Model

Caco-2 cells are derived from colon carcinoma. However, when cultured under specific conditions, the cells become differentiated and polarized such that their phenotype, morphologically and functionally, resembles the enterocytes lining the small intestines that are responsible for nutrient uptake (Sambuy et al., 2004). Caco-2 cells are characterized by tight junctions, microvilli, and a number of enzymes and transporters that are characteristic of such enterocyte. These characteristic structures create a physical and biochemical barrier to the passage of ions and small molecules (Garret et al., 1999).

2.5.3.3. Carotene digestion, absorption and metabolism

Digestion of carotene is the first critical step before absorption and metabolism. The step is characterized by mechanical and enzymatic disruption of food matrices to facilitate release of carotenes and subsequent mixing with lipid droplets to form micelles. The released carotenes are then incorporated into mixed micelles in the duodenum. The mixed micelles consist largely of bile salts, free fatty acids, monoglycerides and phospholipids (Hernell et al., 1990). Incorporation of carotenoids into the mixed micelles is important for their absorption.

Like many other dietary lipids, the absorption of carotene by the enterocytes is believed to occur by simple diffusion. According to the simple diffusion mechanism, the micelles containing carotene migrate through the unstirred water layer to the brush border membrane. The carotene then leaves the micellar structure and diffuses through the membrane into the cytoplasm of enterocytes (Hollander & Ruble, 1978; Scita et al., 1992). Protein mediated transport is also believed to be involved in transport of carotenoids (Reboul et al., 2005; During et al., 2002). According to Reboul et al. (2005) and During et al. (2002), it is possible that some proteins

found in the diet and/or pancreatic biliary secretion bind a fraction of retinol and/or carotenoids and transport them to the brush border membrane. Once absorbed by the enterocyte, a sustained quantity of carotene is not metabolized. A fraction of carotene is cleaved into retinal by the enzyme 15, 15'-dioxygenase which is then converted to retinol and then retinyl esters (Hernell et al., 1990). The quantity of β-carotene metabolized is controlled by a feedback mechanism and cases of VA toxicity from β-carotene have not been reported.

2.5.4. Factors affecting carotenoid bioaccessibility

2.5.4.1. Carotenoid structure

Studies have shown that bioaccessibility of different carotenoids varies widely. Among the different types of carotenoids, lutein is reported to be more bioaccessible while lycopene is the least. This is simply due to the fact that lutein is an oxycarotenoid and therefore more hydrophilic than hydrocarbon carotenoids (Garret et al., 1999). Although different authors have reported contradictory information on bioaccessibility of β-carotene and α-carotene, still β-carotene has high vitamin A value because it has two β-ionone rings. Hence it can be converted into two molecules of vitamin A. OFSP have high proportion of β-carotene, thus provide a good source of provitamin A.

2.5.4.2. Processing

It is well-accepted that cooking or high temperature processing of carotene rich foods disrupts plant cell walls and organelle membranes, facilitating greater access of digestive enzymes to substrates and release of carotenoids for integration into mixed micelles, hence creating high bioaccessibility (Hendren, 2002). Sweetpotato is processed in many ways such as

boiling, roasting and frying prior to consumption. Such processing methods involve maceration and use of heat that result in microstructural changes in the food matrix. The structural changes may also influence digestion and nutrient absorption (Bengtsson et al., 2009; Tumuhimbise et al., 2009). However, a high degree of processing of plant foods also induces isomerization of carotenoids, thus increasing the levels of cis isomers and decreasing total all-trans carotene accessible by enterocytes. For instance, baking is associated with isomerization and degradation of all-trans β-carotene in sweetpotato (Chandler, 1988). Although cis isomers of β-carotene have provitamin A activity, their creation is not desired because their retinol activity equivalence is only one-half that of all-*trans* β-carotene. It is still important to study the effects of various OFSP processing methods on bioaccessibility of β-carotene.

2.5.4.3. Dietary fat and oil

Dietary fat appears to be necessary for the efficient solubilization of lipophilic compounds. Fern´andez-Garc´ıa et al. (2012) suggested that a minimum amount of fat is necessary for digestion and absorption of carotenoids. In previous study, bioaccessibility of β-carotene increased from 0 to 20% with addition of fat/oil to digestion (Pugliese et al., 2014; Ekase et al., 2012). Similarly, Failla et al. (2008) observed a double increase in bioaccessible β-carotene as a result of addition of triglyceride to carotenoids rich salad. This was explained by creation of lipophilic environment that facilitates the transfer of the carotenoids from the food matrix to lipid droplets during the gastric phase. Moreover, the products of lipid hydrolysis modify the physicochemical characteristics of the micelles, a circumstance that may increase the uptake of carotenoids.

It should be noted that not all oils contribute to improved carotenoid bioaccessibility. Addition of orange oil to carotenoid rich food resulted in low bioaccessibility probably because flavored oils do not contain triglyceride components and therefore cannot be hydrolyzed into free fatty acids (Borel et al., 1998). Considering the diverse types of oils used in carotenoid rich food little is known about the type of oil with greatest potential of *in vitro* carotenoid bioaccessibilty. Thus, the current study evaluated effect of oil type on carotene bioaccessibility.

2.5.5. Bioconversion of β carotene into Vitamin A

Carotenoids are important sources of provitamin A for human health but their metabolism is highly misunderstood. Various studies have been implemented trying to understand carotenoids' metabolism and authors came to a conclusion that carotenoids are absorbed in the small intestines in which some parts of the provitamin A are converted to vitamin A while the rest of intact carotenoids are incorporated into chylomicrons and delivered to the liver and then various tissues (Hernell et al., 1990, Erdman et. al, 1993, and Furr & Clark, 1997). The conversion of dietary β-carotene into vitamin A is catalyzed by the enzyme β, β-carotene-15, 15'-dioxygenase (BCMO1) located in intestinal enterocytes (Goodman & Huang 1965, Oslon & Hayaishi, 1965). The central cleavage of β-carotene with BCMO1 from intestinal post mitochondrial fractions produces two molecules of retinal (RAL) which can be reversibly converted to retinol (ROL) and irreversibly oxidized to retinoic acid (RA). BCMO1 only cleaves carotenoids with a non-substituted β-ionine ring thus, making provitamin A the only substrate. Glover and Redfearn (1954) proposed random cleavage of β-carotene at several double bonds in the polyene chain in addition to the 15, 15' double bond. The hypothesis has been supported by several studies that reported formation of β-apo-carotenals (β-apo CALs) from incubation of β-

carotene with a post nuclear fraction of intestinal tissues (Sharma et. al, 1977, Wang et. al, 1991). The possible mechanism of β-carotene cleavage in purified post mitochondrial fraction is demonstrated in Figure 2-3. In a separate study, Yuem et al., (2000) observed formation of RAL and RA in the presence of α-tocopherol while β-apo-CALs and β-apo CAs were formed in the absence of α-tocopherol (Figure 3-3). The study strengthens the importance of α-tocopherol in the bioconversion of β-carotene to vitamin A.

Like bioaccessibility of carotenoids, the major factors that affect carotenoid bioconversion into vitamin A include food matrices, food preparation and fat/lipid content of the meal (Furr and Clark, 1997). The vitamin A value of plant food is determined by the conversion efficiency of provitamin A in food into vitamin A in the body. The conversion rate of dietary β-carotene to VA is relatively low compared to preformed vitamin A from animal origins or from vitamin A supplements that can be absorbed and stored in the body very effectively. Scientific data from human studies reported that the conversion efficiency of dietary β-carotene to vitamin A is in the range of 10-28:1 by weight (Haskell et.al, 2004 and Tang et.al 2005). Based on these data, the Food and Nutrition Board revised the estimated efficiency factor for the conversion of dietary β-carotene to vitamin A from 6:1 by weight (NRC, 1989) to the newer value 12:1 by weight (IOM, 2001). These figures could be revised again, hence should be should be viewed with caution.

Methods to study conversion of β-carotene to vitamin A at physiological dose and dietary intake include; depletion-repletion, changes in concentration of serum vitamin A, changes in whole-body stores of vitamin A or Paired Deuterated Retinal Dilution (DRD) and use of stable isotopes. The challenge of such methods lies in the inability to distinguish between newly formed retinol from body reserves (Tang et al., 2005). The variability among human subjects in

intrinsic conversion of dietary β-carotene to vitamin A is another challenge. In the recent past, *in vitro* methods to determine bioconversion rate of β-carotene have been developed to overcome challenges associated with human studies.

Although several works have reported bioconversion efficiency of provitamin A carotenoid carotene into vitamin A, no report has provided the bioconversion efficiency of carotenoids found in OFSP based products. It is widely known that OFSP is a staple crop in most African countries hence the need to understand the conversion efficiency of provitamin A carotenoids found in OFSP products like *chapati* and porridge.

2.5.5.1. *In vitro* bioconversion of β-carotene into vitamin A

In vitro conversion of β-carotene to vitamin A is facilitated by 15, 15' dioxygenase enzyme (BCMO1). The enzyme has been cloned from small intestines of humans, mouse, chicken and zebrafish (Wyss et.al, 2000). During *in vitro* conversion of β-carotene to vitamin A, all-trans β-carotene extract is solubilized in aqueous solution containing Tween 40 and incubated with an enzyme preparation of known concentration (7.7 $\pm$ 0.01 pH) at 37 ^{o}C for 30 min. The reaction is terminated with addition of formaldehyde and then acetonitrile. This is followed by centrifugation and supernatant subjected to HPLC analysis of retinal (During et.al, 1996, Yeum et.al, 2000). With this method, During et al., (1996) achieved >93% retinal recovery.

2.6 MECHANISM AND FACTORS OF CAROTENE DEGRADATION DURING PROCESSING AND STORAGE

2.6.1. Isomerization and autoxidation

Direct exposure of β-carotene rich food to sunlight initiates isomerization of all-*trans* carotenoid to the cis isomer. The twisting of the carotenoid molecule leads to unpaired spin which readily reacts with oxygen (Mordi, 1993; De Moura et al., 2015). The interaction between cis isomers with oxygen or singlet oxygen causes formation of diradicals. Oxygen attack on either side of the cis bond produces β-carotenyl peroxyl radicals. Further reaction of oxygen/singlet oxygen with peroxyl radical yields the final stable compounds, apocarotenals and apocarotenones (Mordi, 1993). The formation of final stable compounds marks complete loss of β-carotene in food, hence no provitamin A benefits upon consumption. Figure 2-4 illustrates possible mechanism of carotenoid degradation.

Among the carotenoids, xanthophylls are more susceptible due to high degree of unsaturation. Besides autoxidation other mechanisms of carotenoid degradation include heat, and interaction with acid as well as metals (De Moura et al., 2015). Figure 2-5 is a summary of factors causing β-carotene degradation. Control of rapid carotenoid degradation remains a challenge because the compound is so unstable and its degradation is affected by so many factors. Use of yellow light during processing/handling and packaging with aluminum foil laminate paper are some of the ways improvised to minimize carotenoid degradation (De Moura et al., 2015).

2.6.2. Carotenoid losses during storage of dried products

As explained above carotenoid is subject to degradation over a period of time in storage. Significant studies have reported carotenoid losses during storage of dried products. Among the published literature, a study conducted in Kenya reported 50% all-trans β-carotene loss at room temperature (about 25 ºC) in chips from Kakamega and Jonathan varieties whereas it remained steady in sweetpotato chips stored for 3 months in a freezer (-20 ºC) (Kósambo 2004). Storage of dried sweetpotato chips at room temperature in Uganda, resulted in about 70% of total carotenoid loss after four months and this was not dependent on the use of opaque or transparent packaging (Bechoff et al., 2010). However, there are contradictory findings on carotene loss in different packaging materials. Hagenimana et al. (1999) found that total carotenoid loss was reduced by 10% when dried slices from 24 sweetpotato genotypes were stored in opaque paper bags under ambient conditions for 11 months. Hagenimana et al. (1999) further observed that carotenoid losses during storage were higher in flour than dried chips/slices. The high carotenoid loss of flour was related to small particle size that might have increased surface area exposed to oxidation. Thus, it is more sensible to store dried chips/slices and make flour as needed. Carotene retention of various sweetpotato products stored in different packaging is shown in Table 2-3. Still, related to packaging material and β-carotene loss of stored OFSP products, kraft paper is the main packaging material used to store dried chips or flour in Africa. Although carotene losses associated with kraft paper packaging are not well documented, it is obvious that the losses are very high based on the color and due to lack of vacuum sealing of kraft paper. Alternative packaging, like HDPE and AFL are available in Africa and could be evaluated for carotene retention of stored OFSP flour. Stability of carotenoid in stored, dried sweetpotato products is

adversely affected by temperature, light, oxygen and water activity as elaborated in the sections below.

2.6.2.1. Temperature

The influence of storage temperature on carotenoid degradation during storage lies in the induction of isomerization of β-carotene, with low heat intensity causing less damage than high heat intensity. Freeze dried powders from orange peels, sweetpotato and carrot stored at 4, 20, 40 and 45°C showed significant difference in carotenoid content due to effect of temperature (Tang & Chen, 2000; Cinar 2004). During storage of freeze dried red guava (*Psidium guayava* L.) at ambient temperature more pronounced β-carotene losses occurred during the first six months, becoming progressively smaller and almost irrelevant at the end of the period (Nogueir et al. 1978). However, Woolfe (1992) reported no influence of storage temperature (at 0, 7, 14 or 21°C) on the β-carotene content in sweetpotato products. A slight degree of isomerization of β-carotene was noted in the pumpkin puree samples stored at 23°C, but with low concentrations of *cis*-isomers. Provesi et al. (2011) further reported that storage for 180 days did not significantly affect the concentrations of the carotenoids under study. Based on the reviewed literature, high storage temperature and extended storage at ambient temperature negatively affected carotene content of foods. Such losses could be minimized by storing in freezer or refrigerator as well as limiting the storage period in order to preserve the nutrient.

2.6.2.2. Water activity

Scant information is available on the effect of water activity on carotene loss during storage of dried sweetpotato. From the little information that is available Haralampu and Karel

(1983) showed that water activity affected degradation of carotenoid in OFSP dried products. Water activity significantly increased from 0.365 to 0.432 in stored, diced carrot slices during 6 months of storage (Sra et al., 2014). The water activity of stored dried capsicum was related to packaging material. Aluminum foil and HDPE had a non-significant effect on water activity for both stored red and yellow capsicum indicating that they provided barriers against moisture (Swain et al., 2013). The lack of sufficient published information on water activity and carotene loss of stored, dried sweetpotato suggest the need for more research.

2.6.2.2. Light

Carotenoid degradation due to light is mostly due to photo-oxidation. Light increased cis-isomerization of α-carotene, β-carotene and lutein standards compared to samples stored in the dark (Tang & Chen, 2000). Moderate effects of light on β-carotene isomerization compared to temperature have been reported. Chen and Huang (1998), observed that isomerization of β-carotene in hexane reached the same level in 40 min at 70°C in the dark and in 12 hours at –5°C under 2000 lux. Contradictory information is available on the effect of light on carotene loss. Rodriguez and Rodriguez-Amaya (2007) did not observe significant impact of light exposure on β-carotene degradation in the time scale chosen (21 days). The effects of photo-oxidation in carotene rich foods could be minimized during processing by preventing light around the product, and also by dark/opaque packaging during storage.

2.6.2.4. Oxygen

The current school of thought on the mechanism of carotene oxidation with molecular oxygen is that the whole process involves first isomerization of the all-trans to the cis-isomer,

followed by the formation of a diradical, or they may both occur simultaneously and reversibly. Head-space oxygen in the storage bags influences β-carotene content. Samples stored for six weeks under 2% oxygen suffered an average loss of 4.4% of β-carotene more than samples stored under 0% oxygen (Collins et al, 1990). Even greater carotene losses were expected with an extended storage period. High oxygen concentrations were related to high levels of carotenoid degradation during storage in dried sweetpotato flakes (Emenhiser et.al, 1999), in pasteurized mango puree (Vásquez-Caicedo et al., 2007) and in semi-preserved tomato sauces (Baiano et al., 2005). The impermeable packaging to oxygen (laminate) with oxygen absorber was effective at preventing carotenoid degradation through oxidation (Emenhiser et.al, 1999). The high cost and scarcity of laminate paper bags limits their use in sub-Saharan Africa hence kraft paper and LDPE with high oxygen permeability are the available options.

2.6.3. Carotenoid losses during processing

In recent past, several studies have shown that there is significant carotenoid degradation from sweetpotato during preparation. Jaarsveld et al. (2006) studied the true retention (TR) of β-carotene in boiled, and mashed OFSP and found that the true retention of β-carotene varied with the preparation methods (Table 2-5). The β- carotene was retained better when sweetpotato roots were boiled unpeeled, covered with water in a pot with the lid on for 20 min. Boiling of sweetpotato roots caused 20% loss of total carotenoid content while drying of sweetpotato chips reduced the amount of total carotenoid by 30% (Hagenimana et al., 1998). At least 68% of β-carotene per gram fresh weight was retained after boiling or boiling and briefly frying (Berni et al., 2014). The retention of all-*trans*-β-carotene in porridges ranged from 69 to 93% while in *chapatis* it ranged from 70 to 97% (Bechoff et al., 2011). Kean et al. (2008) reported carotene

retention of 52% (yellow maize porridge) and 75% (yellow maize bread). Authors attributed carotenoid loss during processing to isomerization due to high temperature. Isomerization is the main reaction that occurs during thermal processing at atmospheric pressure and at temperatures lower than 100°C. It is reported that 13-*cis* forms at a higher rate than 9-*cis* and 15-*cis* β-carotene during processing of provitamin A rich foods (Chen & Huang 1998; Henry et al., 1998).

Formation of oxidation products from β-carotene, such as epoxides and apo-carotenals, as well as di-*cis* isomers occur under higher temperature, longer time, and higher pressure processing (Mercadante, 2008). Typically, longer thermal processing of provitamin A products is associated with high isomerization of carotenoid due to extended exposure to high temperature. Besides isomerization, Bechoff et al. (2018), explained physical losses such as leaching of carotenes in water experienced at an initial step of processing as factor responsible for carotene degradation in processed products. Although cis isomers have provitamin A activity, their formation during processing is detrimental since their retinal activity equivalent is half that of all-*trans* β-carotene.

2.7. SUMMARY OF ISSUES HIGHLIGHTED BY THE LITERATURE REVIEW

2.7.1. Summary and outcomes

Biofortification of sweetpotato high in provitamin A offers a better and sustainable approach to tackle vitamin A deficiency in sub-Saharan Africa. Significant studies have reported the efficacy of the crop in improving vitamin A status among vulnerable communities. In Africa, OFSP is processed into a variety of products such as bread, biscuits, juice and crisps. Traditional

products such as porridge and *chapati* supplemented with porridge are commonly consumed in Africa hence a good source of provitamin A to tackle vitamin A deficiency.

OFSP flour is the common primary product used in formulation of various products such as porridge and *chapati*. In Kenya OFSP is available on the market. Provitamin A compounds are unsaturated unstable carotenoids easily degraded by light (UV), oxygen, and heat. Consequently, storage of OFSP flour causes carotenoid loss due to the aforementioned factors. Packaging that limits oxygen and light is required for high carotene retention of stored OFSP flour. It is therefore important to understand the effect of packaging material and storage environment on carotene content of stored OFSP flours.

Processing of sweetpotatoes may increase nutrient loss but at the same time increase nutrient bioaccessibility. Carotenoids bioaccessibility is defined as the fraction of carotenoids transferred by food to mixed micelles, therefore becoming accessible for subsequent uptake by intestinal mucosa. After digestion and absorption, β-carotene is cleaved into retinal by the enzyme 15, 15'-dioxygenase and is then converted to retinol and then retinyl esters. Carotenoid bioaccessibility is affected by carotenoid structure, processing, and dietary oil/fat. It is therefore essential to evaluate the effect of OFSP processing and oil type on carotene bioaccessibility to determine product and oil type with highest carotene delivery after digestion. Bioconversion rate of β-carotene into vitamin A in OFPS supplemented products also needs to be investigated.

REFERENCES

Abidin, P. E., Dery, E., Amagloh, F. K., Asare, K., Amoaful, E. F., & Carey, E. E. (2015). Training of trainers' module for orange-fleshed sweetpotato (OFSP) utilization and processing. International Potato Center (CIP); Nutrition Department of the Ghana Health Service, Tamale (Ghana). 32pp

Bechoff, A., Westby, A., Dhuique-Mayer, C., & Tomlins, K. (2010). Effect of drying and storage on the degradation of total carotenoids in orange-fleshed sweetpotato cultivars. Journal of Science of Food and Agriculture, 90: 622–629

Bechoff, A., Poulaert, M., Keith, I., Tomlins, K., Westby, A., Menya, G. Young, S., & Dhuique-Mayer, C. (2011). Retention and bioaccessibility of β-carotene in blended foods containing orange-Fleshed sweetpotato flour. Journal of Agriculture and Food Chemistry, 59:10373–10380

Bechoff, A., Tomlins, K. I., Chijioke, U., Ilona, P., Westby, A., & Boy, E. (2018). Physical losses could partially explain modest carotenoid retention in dried food products from biofortified cassava. PLoS ONE 13(3): e0194402. https://doi.org/

Bengtsson, A., Namutebi, A., Almingera, M. L., & Svanberg, U. (2008). Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. Journal of Food Composition and Analysis, 21:134–143

Bengtsson, A., Alminger, M. L., & Svanberg, U. (2009). In Vitro bioaccessibility of β-carotene from heat-processed orange-fleshed sweetpotato. Journal of Agriculture and Food Chemistry, 57, 9693–9698

Berni, P., Chitchumronchokchai, C., Canniatti-Brazaca, S. G., De Moura, F. F., & Failla, M.L. (2015). Comparison of content and *in vitro* bioaccessibility of provitamin A carotenoids

in home cooked and commercially processed orange fleshed sweetpotato (*Ipomea batatas* Lam). Journal of Plant Foods and Human Nutrition, 70: 1-8

Block, G.; Patterson, B., & Subar, A. (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Journal of Nutrition and Cancer, 18: 1–29

Borel, P., Tyssandier, V., Mekki, N., Grolier, P., Rochette, Y., Alexandre-Gouabau, M.C., Lairon, D., & Aza¨ıs-Braesco, V. (1998). Chylomicron β-carotene and retinyl palmitate responses are dramatically diminished when men ingest β-carotene with medium-chain rather than long-chain triglycerides. Journal of Nutrition, 128:1361–1367

Burri, B. J. (2011). Evaluating sweetpotato as an intervention food to prevent vitamin A deficiency. Comprehensive Reviews in Food Science and Food Safety. Institute of Food Technologist

Britton, G. (1995). Structure and properties of carotenoids in relation to function. FASEB Journal, 9(15): 1551-1558

Chandler, L. A., & Schwartz, S. J. (1988). Isomerization and losses of trans-β-carotene in sweetpotatoes as affected by processing treatments. Journal of Agricultural and Food Chemistry, 36: 29–133.

Chen, B. H., & Huang, J. H. (1998). Degradation and isomerization of chlorophyll and β-carotene as affected by various heating and illumination treatments. Journal of Food Chemistry, 62: 299–307

Cinar, I. (2005). Stability studies on the enzyme extracted sweet potato carotenoproteins. Journal of Food Chemistry, 89: 397-401

Craft, N. E., & Scares Jr, J. H. (1992). Relative solubility, stability, and absorptivity of Lutein and β-carotene in organic solvents. Journal of Agricultural and Food Chemistry, 40(3): 431-434.

Collins, J. L., & Gurkin, S. U. (1990). Effect of storage condition on quality of sweetpotato flour. Tennessee Farm Home Science, 156: 20

Courraud, J., Berger, J., Cristol, J. P., & Avallone, S. (2013). Stability and bioaccessibility of different forms of carotenoids and vitamin A during in vitro digestion. Journal of Food Chemistry, 136(2):871–7

De Moura, F. F., Miloff, A., & Boy, E. (2015). Retention of Provitamin A Carotenoids in Staple Crops Targeted for Biofortification in Africa: Cassava, Maize, and Sweet Potato. Critical Reviews in Food Science and Nutrition, 55:1246–1269

Delgado-Vargas, F., Jiménez, A. R., Paredes-López, O., & Francis, F. J. (2000). Natural pigments: Carotenoids, anthocyanins, and betalains - Characteristics, biosynthesis, processing, and stability. Critical Reviews in Food Science and Nutrition, 40(3): 173-289

During A., Nagao, A, Hoshino C., & Terao J. 1996. Assay of β-Carotene 15, 15'-Dioxygenase Activity by Reverse-Phase High-Pressure Liquid Chromatography. Journal of Analytical Biochemistry, 241: 199–205

During, A., Albaugh, G., & Smith, Jr. J. C. (1998). Characterization of β-Carotene 15, 15'-Dioxygenase Activity in TC7 Clone of Human Intestinal Cell Line Caco-2. Journal of Biochemical and Biophysical Research Communications, 249: 467–474

During, A., Mahmood Hussain, M., Morel, D. W., & Harrison, E. H. (2002). Carotenoid uptake and secretion by CaCo-2 cells: β-Carotene isomer selectivity and carotenoid interactions. Journal of Lipid Research, 43(7): 1086-1095

Emenhiser, C., Watkins, R. H., Simunovic, N., Solomons, N., Bulux, J., Barrows, J., & Schwartz S. J. (1999). Packaging preservation of β-carotene in sweet potato flakes using flexible film and an oxygen absorber. Journal of Food Quality, 22: 63–73

Ekesa, B., Poulaert, M., Davey, M. W., Kimiywe, J., Van den Bergh, I., Blomme, G., & Dhuique-Mayer, C. (2012). Bioaccessibility of provitamin A carotenoids in bananas (*Musa spp*.) and derived dishes in African countries. Journal of Food Chemistry, 133: 1471–1477

El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G., & Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Archives of Biochemistry and Biophysics, 430(1): 37-48

Erdman, J. W., Bierer, T. L., & Gugger, E.T. (1993). Absorption and transport of carotenoids. Journal of Food Science and Human Nutrition, 691: 76-85

Failla, M. L., Thakkar, S. K., & Kim, J. Y. (2009). In vitro bioaccessibility of β-carotene in orange-fleshed sweet potato (*Ipomoea batatas, Lam*). Journal of Agricultural and Food Chemistry, 57: 10922-10927

Food and Agriculture Organization of the United Nations. (1995). Sorghum and Millets in Human Nutrition. Food and Nutrition Series 27; FAO: Rome, Italy

FAOSTAT. Statistical year book 2013. World food and agriculture. http://faostat.fao.org/site/609/DesktopDefault.aspx?PageID=609#ancor. Accessed on October 25, 2018

Fern´andez-Garc´ıa, E., Carvajal-L´erida, I., Jar´en-Gal´an, M., Garrido-Fern´andez, J., P´erez-G´alvez, A., & Hornero-M´endez, D. (2012). Carotenoids bioavailability from foods:

from plant pigments to efficient biological activities. Food Research International 46(2):438–50

Food and Nutrition Board, Institute of Medicine. (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. A report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing committee on the Scientific Evaluation of Dietary Reference Intakes. Washington, DC: National Academy Press

Furr, H. C., & Clark, R. M. (1997). Intestinal absorption and tissue distribution of carotenoids. Journal of Nutrition and Biochemistry, 8:364-77

Garret D. A., Failla M. L., & Sarama R. J. (1999). Development of *in vitro* digestion method to assess carotenoid bioavailability from meals. Journal of Agriculture and Food Chemistry, 47:4301-9

Glover, J., & Redfrean, E. R. (1954). The mechanism of the transformation of β-carotene into vitamin A *in vivo*. Journal of Biochemistry, 58: 15-16

Goodman, D. S., & Huang, H. S. (1965). Biosynthesis of Vitamin A with rat intestinal enzymes. Science, 149(3686):879-880

Hagenimana, V., Carey, E. E. Gichuki, S. T . Oyunga, M. A., &. Imungi. J. K. (1998). Carotenoid contents in fresh, dried and processed sweetpotato products. Journal Ecology of Food and Nutrition, 37: 455-473

Hall, A., Bockett, G., & Nahdy, S. (1998). Sweetpotato postharvest systems in Uganda: strategies, constraints, and potentials. International Potato Center Social Science Department

Haralampu, S. G., & Karel, M. (1983). Kinetic models for moisture dependence of ascorbic acid and β-carotene degradation in dehydrated sweet potato. Journal of Food Science, 48: 1872-1873

Haskell, M. J., Jamil, K. M., & Hassan, F. (2004). Dairy Consumption of Indian Spinach (*Basella alba*) or sweetpotato has a positive effect on total-body vitamin A stores in Bangladesh men. American Journal of Clinical Nutrition, 4:80:705-14

Heaney R. P. (2001). Factors influencing the measurement of bioavailability, taking calcium as a model. Journal of Nutrition, 131(4):1344–8

Hedr´en, E., Diaz, V., & Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. European Journal of Clinical Nutrition, 56(5):425–30

Henry, L., Catignani, G., & Schwartz S. (1998). Oxidative degradation kinetics of lycopene, lutein and 9-cis- and all-trans b-carotene. Journal of American Oil and Chemistry, 75:823–829

Hernell, O., Staggers, J. E., & Carey, M. C. (1990). Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Journal of Biochemistry, 2041-2056

Hollander, D., & Ruble, P. E. (1978). β-carotene intestinal absorption: bile, fatty acid, pH, and flow rate effects on transport. American Journal of Physiology, 235: 686-691.

International Potato Center. About Vitamin A for Africa. http://web.archive.org/web/20081229230535/http://www.cipotato.org/vitaa/about_vitaa. Accessed on October 25, 2018

Jaarsveld, P. J. V., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., & Benade, A. J. (2005). Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. American Journal of Clinical Nutrition, 81: 1080-1087

Jaarsvelda P. J. V, Maraisa, D. W, Harmsea, E., Nestel, P., & Rodriguez-Amaya D. B. (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. Journal of Food Composition and Analysis, 19 (2006): 321–329

Kean, E. G., Hamaker, B. R., & Ferruzzi, M. G. (2008). Carotenoid bioaccessibility from whole grain and degermed maize meal products. Journal of Agriculture and Food Chemistry, 56: 9918–9926

Kósambo, L. (2004). Effect of storage and processing on all-trans β-carotene content in Orange-freshed sweetpotato (Ipomoea batatas (L.) Lam) roots and its products. CIP Funded Research Project: Annual Report (July 2003 – June 2004). Kenya Industrial Research and Development Institute. Nairobi. 11pp

Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F., & Tschirley, D. (2007). A food based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. Journal of Nutrition, 137: 1320-1327

Ndolo, P. J., Nungo, R. A., Kapinga, R. E., & Agili, S. (2007). Development and promotion of orange-fleshed sweetpotato varieties in Western Kenya. Proceedings of the 13th ISTRC Symposium, 689 – 695

Maiani, G., Castón, M. J. P., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D., & Schlemmer, U. (2009). Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Journal of Molecular Nutrition and Food Research, 53(2): 194-218

Marangoni, Jnr L., Itob, D., Ribeiroc, S. M. L., Gomes, D. S., & Alves, R.M.V. (2018). Stability of β-carotene rich sweet potato chips packed in different packaging systems. Journal of Food Science and Technology, 92 (2018): 442–450

Mercadante, A. (2008). Carotenoids in foods: Sources and stability during processing and storage. In: Socaciu C. (ed.) Food Colorants: Chemical and functional properties. CRC Press, Boca Raton, USA, pp 213–240

Martin, K. R., Failla, M. L., & Smith Jr, J. C. 1996. β-carotene and lutein protect HepG2 human liver cells against oxidant- induced damage. Journal of Nutrition, 126(9): 2098-2106

Minekus, M., Smeets-Peeters, M., Havenaar, R., Bernalier, A., Fonty, G., Marol-Bonnin, S., Alric, M., Marteau, P., & Huis In't Veld, J. H. J. (1999). A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products. Journal of Applied Microbiology and Biotechnology, 53(1): 108-114

Mordi, R. C. (1993). Mechanism of β-Carotene Degradation. Biochemical Journal, 292 (1): 310-312

National Research Council. (1989). Food and Nutrition Board. Recommended Dietary Allowance. Washington, DC: National Academy Press

Nogueira, J. N., Soybihe Sobrinho. J., Vencosvsky R., & Fonseca, H. (1978). Effect of storage on the levels of ascorbic acid and beta-carotene in freeze dried red guava (*Psidium guayava L.*). Archivos Latinoamericanos de Nutrición, 28(4):363-77

Olson, J. A., & Krinsky, N. I. (1995). Introduction: The colorful, fascinating world of the carotenoids: Important physiologic modulators. FASEB Journal, 9(15): 1547-1550

Oslon, J. A., & Hayaishi, O. (1965). Proceedings of the National Academy of Science in United States of America, 54:1364-1370

Oshima, S., Ojima, F., Sakamoto, H., Ishiguro, Y., & Terao, J. (1996). Supplementation with carotenoids inhibits singlet oxygen-mediated oxidation of human plasma low-density lipoprotein. Journal of Agricultural and Food Chemistry, 44(8), 2306-2309

Porrini, M., Riso, P., Brusamolino, A., Berti, C., Guarnieri, S., & Visioli, F. (2005). Daily intake of a formulated tomato drink affects carotenoid plasma and lymphocyte concentrations and improves cellular antioxidant protection. British Journal of Nutrition, 93(1), 93-99

Provesi, J. G., Dias, C. O., & Amante, E. R. (2011). Changes in carotenoids during processing and storage of pumpkin puree. Journal of Food Chemistry, 128 (2011): 195–202

Pugliese, A. O'Callaghan, Y., Tundis, R., Galvin, K., Menichini, F., O'Brien, N., & Loizzo, M. R. (2013). The effect of domestic processing on the content and bioaccessibility of carotenoids from chili peppers (*Capsicum*species). Journal of Plant Foods and Human Nutrition, 69, 8–17

Reboul, E., Richelle, M., Perrot, E., Desmoulins-Malezet, C., Pirisi, V., & Borel, P. (2006). Bioaccessibility of carotenoids and vitamin E from their main dietary sources. Journal of Agricultural and Food Chemistry, 54(23**)**: 8749-8755

Rodriguez Amaya D. B., & Kimura M. 2004. HarvestPlus Handbook for Carotenoid Analysis. Copyright HarvestPlus, Washington DC

Rodriguez E. B., & Rodriguez-Amaya D. B. (2007). Formation of apocarotenals and epoxycarotenoids from β-carotene by chemical reactions and by autoxidation in model systems and processed foods. Journal of Food Chemistry, 101 (2007) 563–572

Sambuy, Y. (2004). The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Journal of cell Biology and Toxicology, 21(1):1-26

Scita, G., Aponte, G. W., & Wolf, G. (1992). Uptake and cleavage of β-carotene by cultures of rat small intestines and human lung fibroblast. Journal of Nutrition Biochemistry, 3: 118-123

Siems, W., Wiswedel, I., Salerno, C., Crifo, C., Augustin, W., Schild, L., Langhans, C. D, & Sommerburg, O. (2005). Carotene breakdown products may impair mitochondrial functions potential side effects of high-dose β-carotene supplementation. Journal of Nutritional Biochemistry, 16, 385–397

Sharma, R. V., Mathur, S. N, Dimitrovskii, A. A., Das, R. C., & Ganguly, J. Studies on Metabolism of β-Carotene and apo-β-carotenoids in Rats and Chickens. Journal of Biochemistry and. Biophysics, 486: 183-194: 1977

Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M., & Grusak, M. G. (2005). Spinach or carrot can Supply Significant Amounts of Vitamin A as Assessed by Feeding with Intrinsically Deuterium-labeled Vegetables. American Journal of Clinical Nutrition, 82:821-8

Tang, Y.C., & Chen, B. H. (2000). Pigment change of freeze-dried carotenoid powder during storage. Journal of Food Chemistry, 69: 11-17

Trancoso-Reyes, N., Ochoa-Martínez, L.A., Bello-Pérez, L.A., Morales-Castro, J., Estévez-Santiago, R., &, Olmedilla-Alonso, B. (2016). Effect of pre-treatment on physicochemical and structural properties and the bioaccessibility of β-carotene in sweet potato flour. Journal of Food Chemistry, 200: 199–205

Tumuhimbise G.A., Namutebi A. and Muyonga J.H. 2009. Microstructure and in vitro beta carotene bioaccessibility of heat processed orange fleshed sweet potato. Journal of Plant Foods and Human Nutrition, 64:312-318

Sra, S. K., Sandhu, K. S. & Ahluwalia, P. 2014. Effect of treatments and packaging on the quality of dried carrot slices during storage. Journal of Food Science and Technology, 51(4), 645–654

Swain, S. Samuel D. V. K & Abhijit K. A. R. (2013). Effect of packaging materials on quality characteristics of osmotically pretreated microwave assisted dried sweet pepper (Capsicum annum L.). Journal of Food Processing Technology, 2013: 4-9

Vásquez-Caicedo A. L., Schilling, S., Carle R., & Neidhart, S. (2007). Effects of thermal processing and fruit matrix on β-carotene stability and enzyme inactivation during transformation of mangoes into puree and nectar. Journal of Food Chemistry, 102:1172–1186

Varzakas T., & Kiokias, S. (2016). HPLC Analysis and Determination of Carotenoid Pigments in Commercially Available Plant Extracts. Current Research in Nutrition and Food Science Vol. 4(Special Issue 1), 01-14 (2016)

Wang, X. D., Tang, G., Fox, J.Krinsky, N. I., & Rusell, M. (1991). Enzymatic conversion of β-carotene into β-apo-carotenals and retinoids by human, monkey, ferret, and rat tissues. Archives of Biochemistry and Biophysics, 258: 8-16

World Health Organization. (1995). Global prevalence of vitamin A deficiency. Geneva, World Health Organization, 1995 (WHO/NUT/95.3)

World Health Organization and Food and Agriculture Organization. (2004). Vitamin and Mineral Requirements in Human Nutrition. WHO, Geneva

Woolfe, J. A. (1992). Post-harvest procedures: Sweet Potato an Untapped Food Source. Cambridge, UK: Cambridge University Press. 643pp

World Health Organization. (1996). Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating intervention programs. Geneva, (WHO/NUT/96.10; http://whqlibdoc.who.int/hq/1996/ WHO_NUT_96.10.pdf. Accessed on December 28, 2015

World Bank. 2009. Nutrition at a Glance-Kenya. http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2013/05/10/000333037_20130510155529/Rendered/PDF/771750BRI0Box00IC00Kenya0April02011.pdf. Accessed on December 28, 2015

World Bank. 2009. Nutrition at a Glance. http://siteresources.worldbank.org/NUTRITION/Resources/281846-1271963823772/Malawi.pdf. Accessed on December 28, 2015

Wyss, A. Wirtz, G. Woggon, W. Brugger, R. Wyss, M. Friedlein, A., Bachman, H., & Hunziker, W. (2000). Journal of Biochemical and Biophysical Research Communications, 271:334-336.

Yonekura, L., & Nagao, A. (2007). Intestinal absorption of dietary carotenoids. Journal of Molecular Nutrition and Food Research, 51(1): 107-115

Yeum, K. J. Ferreira dos A. A. L., Smith, D., Krinsky N. I., & Russell, R. M. (2000). The effect of α-Tocopherol on the oxidative cleavage of β-Carotene. Journal of Free Radical Biology and Medicine, 29(2):105-14

Table 2-1. Estimates of clinical and subclinical Vitamin A deficiency in preschool children by WHO

Region	Clinical (millions)	Subclinical (severe and moderate) (millions)	Prevalence (%)
Africa	1.04	52	49
The Americas	0.06	16	20
South-East Asia	1.45	125	69
Europe	NA	NA	NA
Eastern Mediterranean	0.12	16	22
Western Pacific	0.13	42	27
Subtotal	2.80	251	
Total		254	

Source: Global prevalence of vitamin A deficiency. Geneva, World Health Organization, 1995 (WHO/NUT/95.3)

Table 2-2. Estimated mean requirement and safe intake level of vitamin A by group

Group	Mean requirement (µg RE/day)	Recommended safe intake (µg RE/day)
Infants and children		
0–6 months	180	375
7–12 months	190	400
1–3 years	200	400
4–6 years	200	450
7–9 years	250	500
Adolescents,		
10–18 years	330–400	600
Adults		
Females,		
19–65 years	270	500
65+ years	300	600
Males,		
19–65 years	300	600
65+ years	300	600
Pregnant women	370	800
Lactating women	450	850

Source: Requirements of vitamin A, iron, folate and vitamin B12. Report of a Joint FAO/WHO Expert Consultation. Rome, Food and Agriculture Organization of the United Nations, 1988 (FAO Food and Nutrition Series, No. 23)

Table 2-3. Effect of OFSP processing on All-*trans* β-carotene bioaccessibility

Product	**Process**	**All-trans β-carotene bioaccessibilty**	**Reference**
Chapati	Pan roasted with oil	73%	Bechoff et al. (2011)
Mandazi	Deep fried	49%	Bechoff et al. (2011)
Porridge	Boiled	16%	Bechoff et al. (2011)
Boiled roots	Homogenization, with oil addition (HOM)	50%	Bengtsson et al. (2010)
Boiled roots	Pureeing with oil addition (BOL)	16%	Bengtsson et al. (2010)
Porridge	Oil addition to flour then cook porridge (POB)	48%	Bengtsson et al. (2010)
Porridge	With oil addition (POA)	31%	Bengtsson et al. (2010)
Flour	Steam treated		Trancoso-Reyes et al. (2016)
	2 min	22.7	
	4 min	42.8	
	6 min	30.1	
Flour	Microwave treated		Trancoso-Reyes et al. (2016)
	2 min	15.1	
	4 min	17.4	
	6 min	27.1	

Table 2-4. Effect of storage and packaging material on β-carotene retention of sweetpotato products

Product	**Packaging material**	**Duration (days)**	**B-Carotene retention**	**Reference**
Sweetpotato flakes	Nylon laminate with vacuum	120	84%	Emenhiser, et al. (1998)
Sweetpotato flakes	Nylon laminate with vacuum	240	65%	Emenhiser, et al. (1998)
Sweetpotato flakes	Nylon laminate with head space	120	70%	Emenhiser, et al. (1998)
Sweetpotato flakes	Nylon laminate with head space	240	52%	Emenhiser, et al. (1998)
Sweetpotato chips	PET/Al/LDPE, BOPP with oxygen scavenger	207 days	90%	Marangoni Júnior et al. (2018)
	Metalized BOPP and BOPP/ with oxygen scavenger	207 days	83%	Marangoni Júnior et al. (2018
	Metalized BOPP with oxygen scavenger	207 days	80%	Marangoni Júnior et al. (2018

PET (polyester), Al (aluminum foil), BOPP (biaxially oriented polypropylene)

Table 2-5. Effect of processing on β-carotene retention of OFSP products

Product	Process	B-carotene retention	Reference
Porridge	Boiling	69 – 93%	Bechoff et al. (2011)
Chapati	Pan roasting with oil	79 – 97%	Bechoff et al. (2011)
Boiled sweetpotato	Roots covered with water, lid on, 20 min	92%	Jaarsveld et al. (2006)
Boiled sweetpotato	Roots covered with water, no lid, 30 min	88%	Jaarsveld et al. (2006)
Boiled sweetpotato	Half covered with water, lid on, 20 min	83%	Jaarsveld et al. (2006)
Fermented sweetpotato	Lactic acid fermentation with brine	93.97%	Bernard et al. (2014)
Dried slices	Fresh oven dried, 57 ^{o}C	88.2%	Bengtsson et al. (2008)
Dried slices	Fresh solar dried, 45/63 ^{o}C	91.1%	Bengtsson et al. (2008)
Dried slices	Fresh open-air-sun dried, 30/52 ^{o}C	83.3%	Bengtsson et al. (2008)

Figure 2-1. Commercial OFSP products (chips, juice, cookies, and flour) produced in Africa

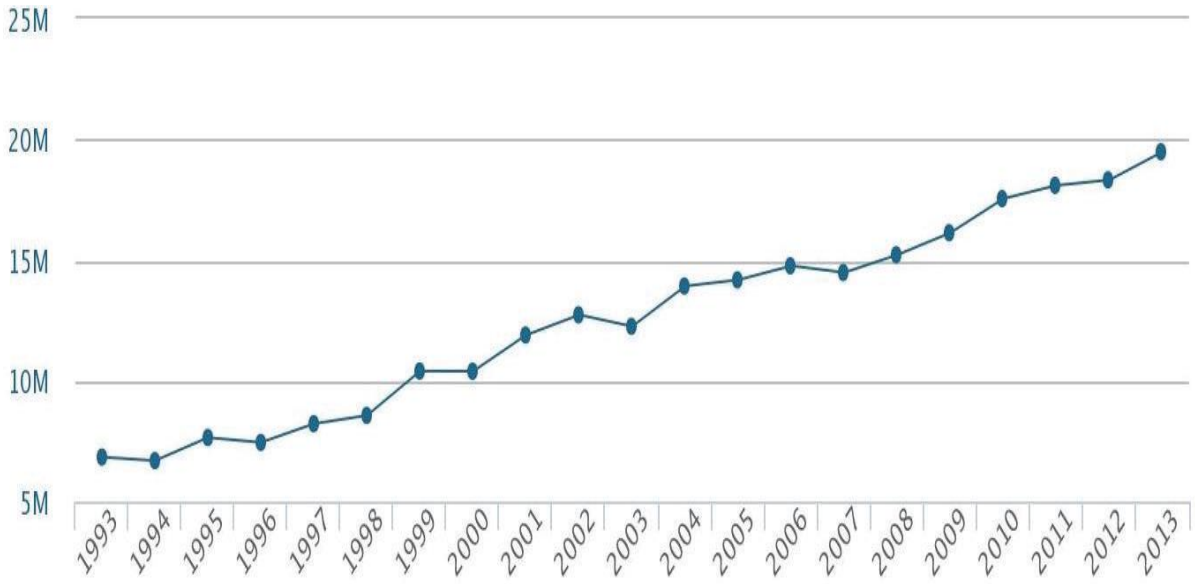

Figure 2-2. Sweetpotato production trend in Africa 1990-2013 (million tons). Source: FAOSTAT

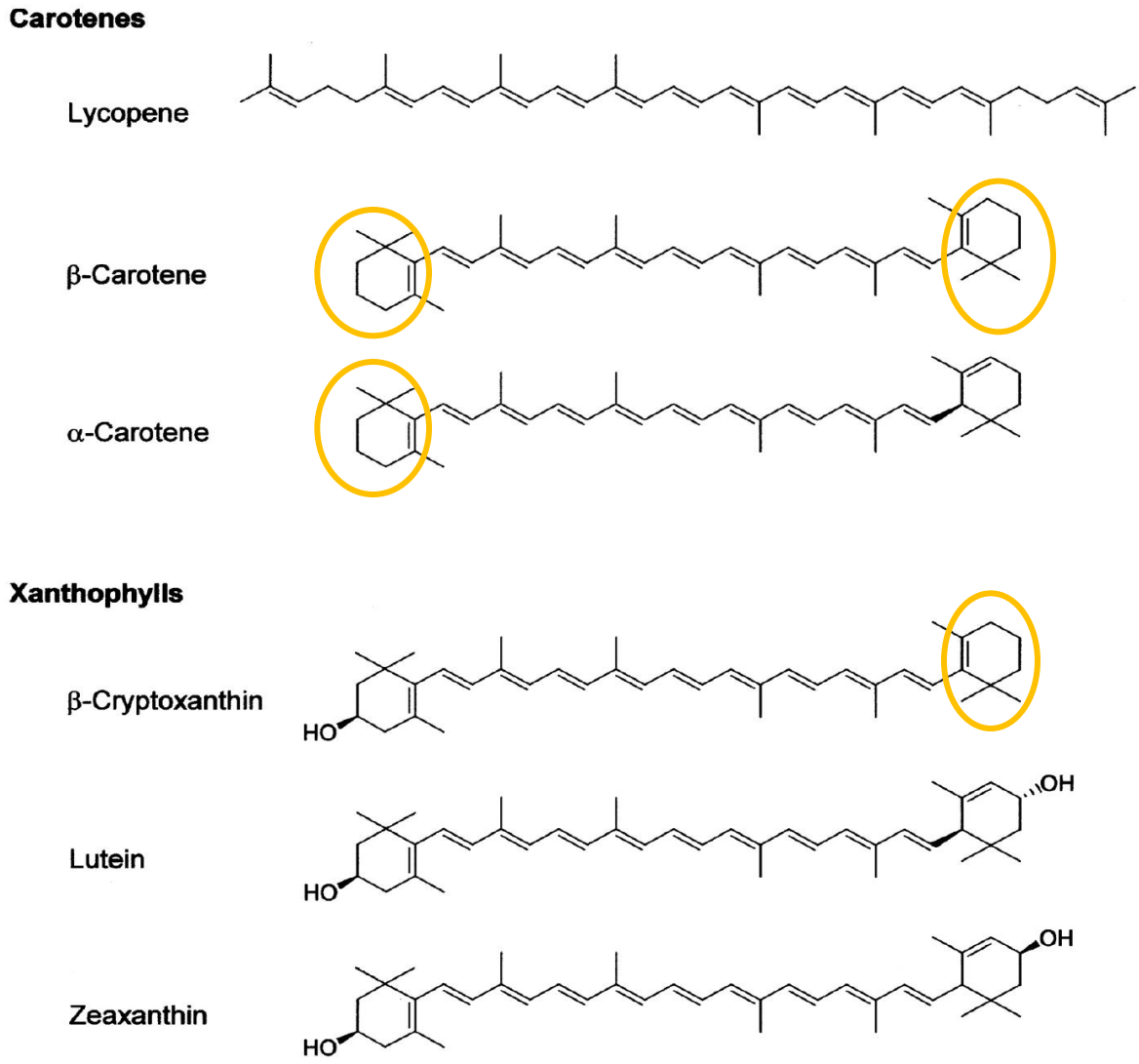

Figure 2-3. Chemical structures of Major Carotenoids Found in Human Plasma. Source: Yonekura and Nagao. 2007

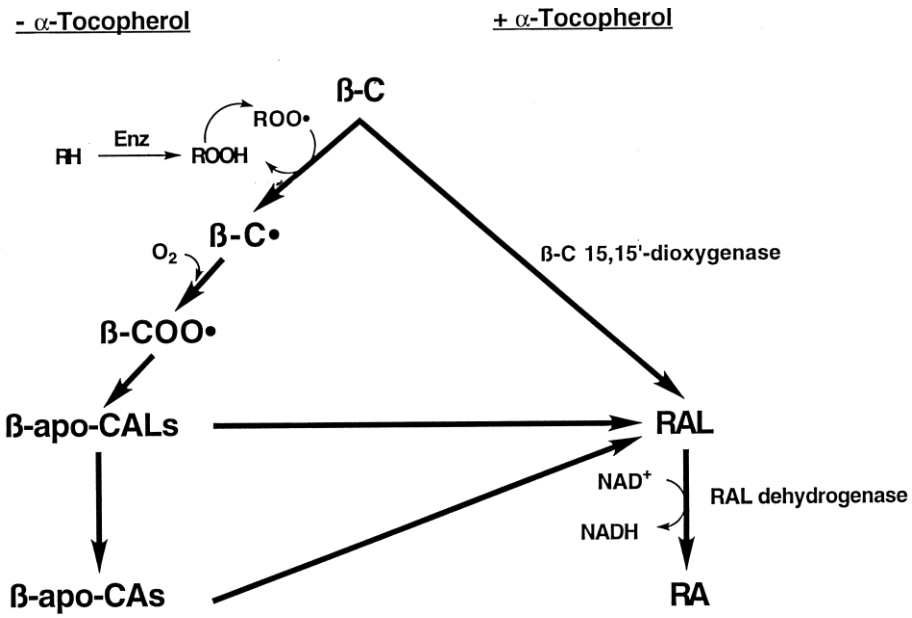

Figure 2-4. Possible mechanism of β-carotene cleavage in a purified, postmitochondrial fraction. B-C= β-carotene; α-T = α-tocopherol; RH = fatty acid; ROO• = hydroperoxyl radical; β-C• = β-carotene alkyl radical; β-COO• = β-carotene peroxyl radical; β-apo-CALs = β-apo-carotenals; β-apo-Cas = β-apo-carotenoic acids; RAL = retinal; RA = retinoic acid. Source Yeum et al., 2000.

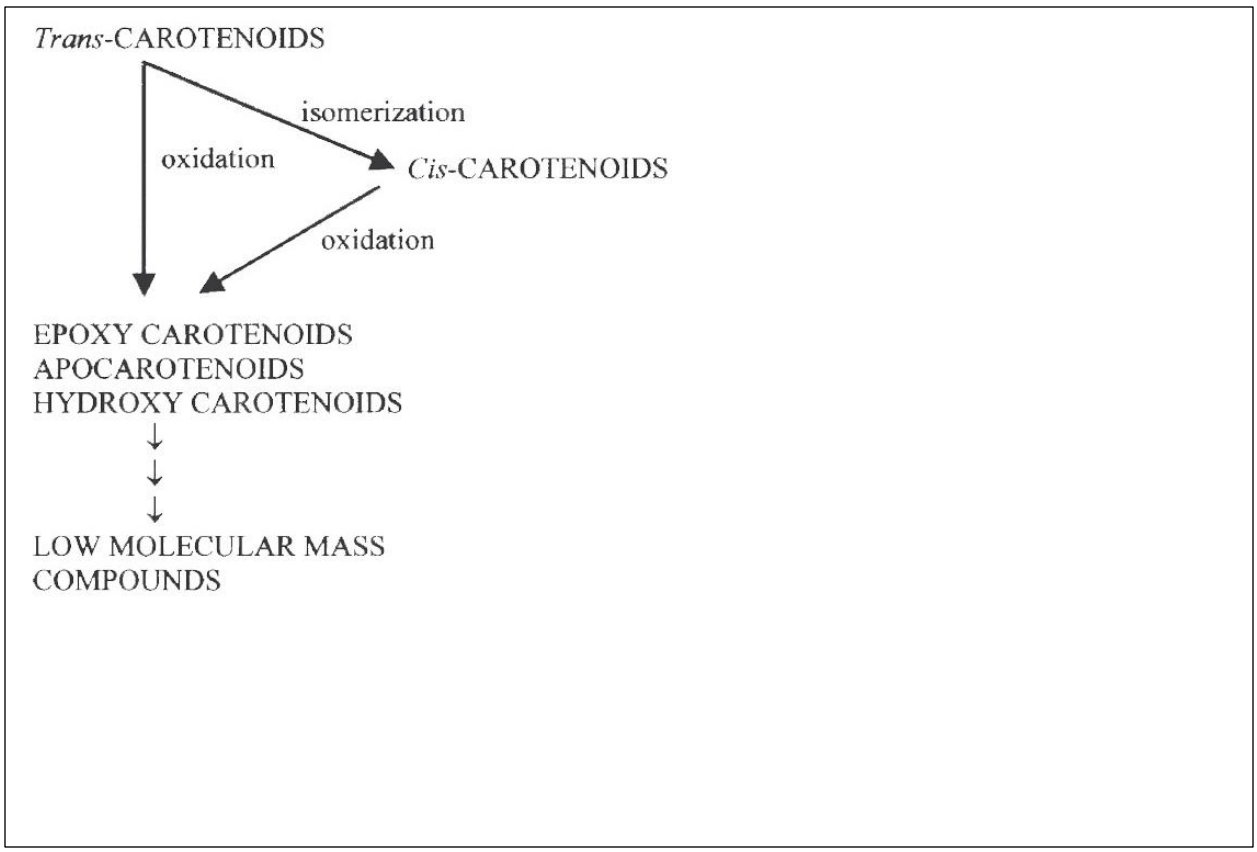

Figure. 2-5. Possible scheme of carotenoid degradation. Source: Rodriguez-Amaya and Kimura, 2004

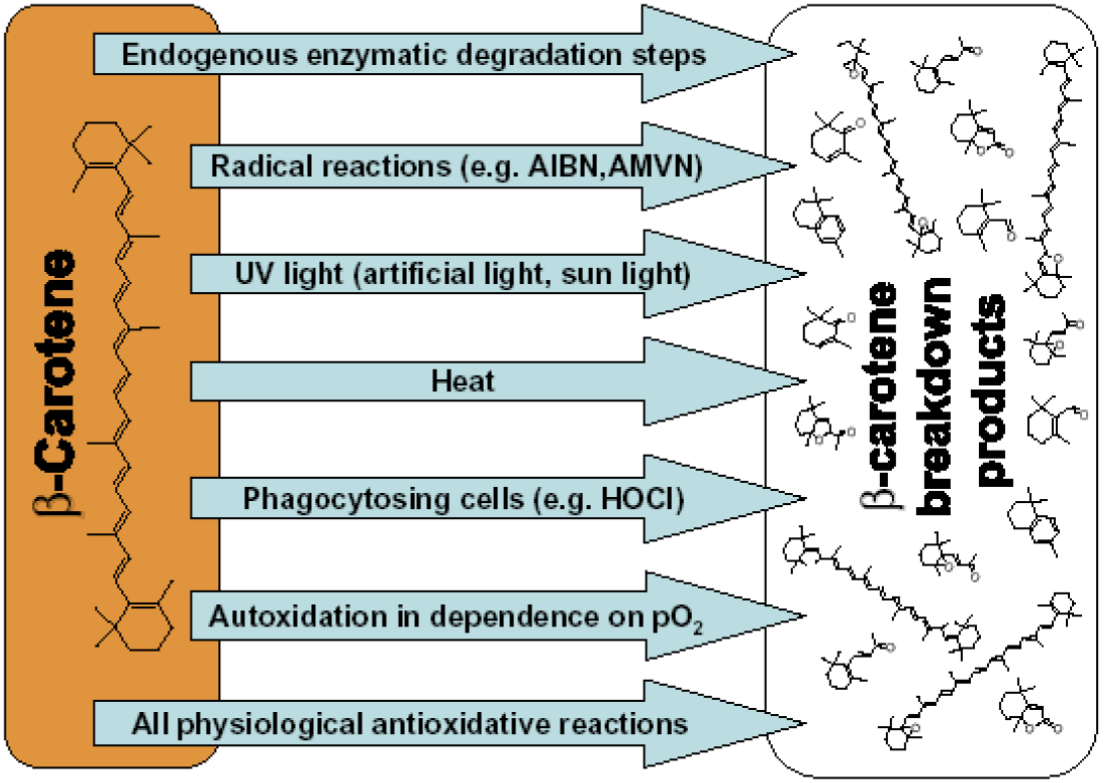

Figure 2-6. Formation of different. Formation of different carotenoid breakdown products from one precursor compound such as β-carotene. Siems et al., 2005.

CHAPTER 3.

EFFECT OF STORAGE AND PACKAGING MATERIALS ON COLOR AND CAROTENOID CONTENT OF ORANGE-FLESHED SWEETPOTATO FLOURS

Effect of Storage and Packaging Materials on Color and Carotenoid Content of Orange-Fleshed Sweetpotato Flours

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Abstract

The loss of carotenes during storage of orange-fleshed sweetpotato (OFSP) flours is a major issue. This study evaluated the effect of storage and packaging materials on carotene content, color and water activity of OFSP flours. Flours from Vita and Kabode OFSP genotypes were packed in aluminum foil laminate (AFL), high density polyethylene and kraft paper and stored under light and dark conditions for 4 mo. Results showed significant carotenoid losses ($P < 0.001$) and color value changes ($P < 0.05$) in stored OFSP flours under both light and dark storage conditions. The highest carotenoid loss was found in flours packed in kraft paper (59.33%) while AFL (29.88%) was the least. A significant ($P < 0.01$) increase in water activity was observed in all packed samples regardless of storage environment. Therefore, the study suggests AFL as the best packaging material for stored OFSP flour due to the low loss of carotenes.

Key words: Sweet potato flour, Storage, packaging material, Color, Carotenoid

3.0. Introduction

Sweetpotato is an important food crop in Eastern and Southern Africa. It plays an important role as a food security crop, providing most of the dietary carbohydrate in South-East Africa especially in times of little rainfall because of its ability to tolerate drought (Hagenimana & Owori, 1996). High nutrient level such as energy, fiber, minerals, vitamins and antioxidants like phenolic acids, tocopherols and β-carotene have tremendously increased the popularity of sweetpotato (Teow et al. 2007). OFSP are particularly important because of high β-carotene content, a precursor for vitamin A, hence, a good and sustainable alternative for vitamin A deficiency mitigation.

In South-East Africa, traditional methods of OFSP utilization include boiling, roasting and drying (Hall et al., 1998). Boiled sweetpotato are taken as part of breakfast, snack or as main meal during lunch or dinner (Woolfe, 1992). Dried chips are milled into flour and used to make porridge, doughnuts, and flat bread (*chapati*) among others (Nungo et al., 2000; Woolfe, 1992). Elsewhere, OFSP powder is used as thickener or gelling agent to impart the desired texture in various products while enhancing the nutritional value (Ray & Tomlins, 2010). Although OFSP flour is associated with the high loss of carotenoids during storage, it is still a main raw material for product development both at local and commercial levels. Rodriguez-Amaya et al. (2011) emphasized the need to optimize the processing and storage conditions to minimize the loss of carotenoids in OFSP flour. The loss of carotenoids in dried OFSP products, like flour and chips, are mostly related to drying method, light, oxygen, water activity, and packaging material. No significant difference in carotenoid loss was observed when dried OFSP chips were dried under open air, oven, and solar driers. Total loss of carotenoids ranged from 16% to 33% with different drying techniques (Bechoff et al., 2009). The slight difference in carotenoid loss was attributed to exposure to direct

sunlight during the open air drying method (Bechoff et al., 2009). Compared to dried chips, the high loss of carotenoids during storage were reported in OFSP flour (Hagenimana et al., 1999). The authors attributed high carotenoid loss of flour was due to the small particle size increasing the surface area exposed to oxygen, hence, high carotenoid losses through oxidation. Lavelli et al. (2007) reported water activity of between 0.31 and 0.54 as having better carotene retention on freeze dried carrots stored at 40^{o}C. These studies suggested a need to explore means of minimizing the loss of carotenoids in stored OFSP flour.

Packaging of OFSP flour is crucial to minimize carotenoid loss in storage. A good packaging material for flour/chips should protect it against light, oxygen and moisture. Packaging materials that are laminate, metallized, coated or aluminum foil are effective at protecting against light, moisture, and oxygen. Where possible, oxygen can be eliminated by creating a vacuum, flushing with inert gas such as nitrogen, or using an oxygen absorber. Sweetpotato flour was packaged and sealed in different forms of packaging such as porcelain and glass jars, tin cans, kraft paper bags, cotton bags, and polyethylene bags (van Hal, 2000) to protect against the aforementioned factors. No change in color was reported when sweetpotato flour was stored in thick gauge white polythene bag, possibly high density polyethylene (HDPE) compared to flour stored in enamel cans, plastic cans or calico bags (Tewe et al., 2003). In a separate study, Bechoff et al. (2010a) reported 83.7% carotenoid loss when dried OFSP chips of different thickness were stored traditionally in jute bags in a mud house for 4 mo.

Although it is general knowledge that prolonged storage of OFSP flour results in high carotenoid degradation, there is a lack of information on the effect of storage conditions and packaging materials on carotenoid retention of OFSP flour. This study was undertaken to evaluate the effect of storage conditions and packaging materials on color, water activity and carotenoid

content of OFSP flours during a storage period of 4 mo. Storage period with highest carotene degradation was also identified. The study evaluated aluminum foil laminate (AFL), HPDE, and Kraft paper on carotenoid retention of OFSP flour made from Vita and Kabode genotypes stored in dark and light conditions.

3.1. Materials and Methods

3.1.1. Source of OFSP roots and flour processing

OFSP flour was produced at Organi Ltd in Ringa, Kenya. Two OFSP genotypes, Vita and Kabode, were harvested 3.5 mo after planting for flour production. OFSP flour was produced following methods described by Abidin et al. (2015) with some modifications. The peeling step of flour production was skipped because OFSP flour is traditionally produced without peeling in Kenya. In brief, sweetpotato roots were sorted, cleaned and trimmed. The roots were washed again and then sliced into 2-mm thick slices by a chipper. The slices were dried in a solar drier for 4 d to 8% moisture content and then milled by hammer mill into flour. The external average temperature and relative humidity during drying were 32°C and 54%, respectively. Temperature and relative humidity inside solar dryer were not recorded due to lack of data logger at the facility.

3.1.2. Packaging and storage

Three types of packaging materials namely Kraft paper (VIP Mills Ltd, Nairobi, Kenya, HDPE (General Industries Ltd, Nairobi, Kenya), and AFL (Packaging Industries Limited, Nadume Road, Nairobi, Kenya) were evaluated for their effect on carotene retention in relation to color. The thickness for HDPE and AFL was 80 and 90 microns respectively. Other packaging material properties like density were not provided by the supplier. Hundred gram flour from Vita and

Kabode genotypes were packed in the aforementioned pouches and stored under light and dark conditions. The light storage of flour was included to simulate home storage condition. Vacuum sealing was applied to HDPE and AFL while Kraft paper had air removed manually and then glued. The two sets of experiments (dark and light) were replicated twice in a factorial experiment. The storage study was conducted for 4 mo and sampling for carotenoid content, moisture content, color, and water activity was done weekly for the first mo, then biweekly for second mo and monthly for the last 2 mo. Temperature and relative humidity were recorded with a data logger (Extech instruments, 9 Townsend W, Nashua, NH 03063, USA) during the entire storage period.

3.1.3. Carotenoid extraction of OFSP flour

Procedure described by Muzhingi et al. (2008) was followed for carotenoid extraction with minor modifications. About 0.6 g of flour was weighed into an extraction tube and 5 mL of methanol was added. The sample was vortexed and incubated at 70°C for 10 min. After incubation, the sample was vortexed again and centrifuged at 3000 rpm for 5 min. The upper phase was transferred into a 25 mL volumetric flask and the residue was extracted four times with 5 mL Tetrahydrofuran (THF). The final volume of the extract was adjusted to 25 mL with THF. Exactly 2 mL of extract was purified with 2 mL water, 4 mL hexane and 1 mL methanol, then centrifuged at 3000 rpm for 5 min. The upper phase was transferred into another test tube and evaporated to complete dryness under liquid nitrogen. The dried extract was reconstituted with 2 mL of methanol:THF solution (85:15 v/v). About 1 mL of sample was transferred to HPLC vial for analysis. Carotenoid extraction was done under yellow light conditions to minimize degradation due to white light.

3.1.4. HPLC analysis of carotenoid

Carotenoids were analyzed by reversed phase HPLC using a Waters 9562 system equipped with auto sampler injector, degasser, pump and Waters 9562-UV-visible photodiode array detector operating at 450 nm (Waters Corporation, Milford, MA). Separations were carried out on a 3-µm, 150 x 3.0 mm, Semibore column (YMC, Wilmington, NC). The isocratic mobile phase consisted of methanol:methyl tert-butyl ether:water (85:12:, v/v/v, 1.5% ammonium acetate (Phase A), 8:90:2, v/v/v, 1% ammonium acetate (Phase B). The flow rate was 0.4 mL/min and injection volume of the sample was 30 µL. Standard curves of pure all-trans β-carotene, 13-cis β-carotene and 9-cis β-carotene were used to quantify the carotenoids. Total carotenoid content was obtained as the summation of the individual carotenes.

3.1.5. Color measurement

Color was measured using a Lovibond LC100 Spectrophotometer (Wilford Industrial Estate, Ruddington Lane, Nottingham). Flour color was described based on L*, a* and b* where L* is a measure of lightness, a* defines components on the red–green axis, and b* defines components on the yellow-blue axis. Color images were taken at three different points per sample and mean was calculated for the parameters. In this study a* was correlated with β-carotene content.

3.1.6. Moisture content determination

Moisture content was determined following AOAC method (AOAC, 1984) on the same day as carotenoid analysis. Five gram of sample was weighed into crucible and dried in the oven

at 105°C until constant weight was achieved. Moisture content was determined as the difference between initial and final weight of sample expressed as a percentage.

3.1.7. Water activity measurement

Water activity of flour was measured with an Aqualab instrument (Decagon Devices Inc., Hopkin Court, Pullman, WA). Calibration was done with LiCL at 0.5 a_w and NaCl at 0.7 a_w.

3.1.8 Statistical analysis

The statistical analysis of data was performed by one way ANOVA using Genstat version 6.0 to determine significant differences among the treatments. The correlation coefficients and their probability levels were obtained from linear regression analysis. Significance differences among treatments was obtained by Tukey's HSD multiple rank test at $P < 0.05$. Data is presented as mean $\pm$ standard error mean (SEM).

3.2. Results and discussion

3.2.1. Identification and quantification of carotenoids

All-trans β-carotene and its isomers, 13-cis β-carotene and 9-cis β-carotene, were the main pro-vitamin A carotenoids found in the sweetpotato flours (Figure 3-1). Basing on the peak area, it is clear from Figure 3-1 that all-trans β-carotene was the most predominant carotenoid found in the products. Vita roots had the total carotenoid content of 22.97$\pm$1.00 mg/100 g dry weight (DW), which was not significantly ($P > 0.05$) different from the value for Kabode roots (21.18$\pm$0.55 mg/100 g, DW). Processing of OFSP roots into flour resulted in significant loss of total carotenoid level. After drying, total carotenoid content of Vita flour was 17.21$\pm$0.69 mg/100 g (DW)

representing 25.08% carotenoid loss while Kabode flour was 15.36±0.13 mg/100 g DW representing 27.48% carotenoid loss.

A slight decrease in 13-cis and 9-cis isomer contents was observed when sweetpotato roots were dried and milled into flour and during the entire storage period (data not shown). Achir et al. (2014) also reported 13-cis and 9-cis isomers as the major isomers of β-carotene degradation during storage of OFSP flour. The current findings are in contradiction to previous findings that indicated increased formation of β-carotene isomers under stressful conditions such as heating, UV exposure and storage (Chandler & Swartz, 1988). Bechoff et al. (2009) reported similar contents of β-carotene isomers before and after drying OFSP chips under hot air, solar drier and direct sun. The amount of isomers formed is related to heat and processing time (Doering et al., 1995). Hiranvarachat et al. (2008) showed that a minimum of 5 h at constant temperature (60°C) favored formation of 13-cis β-carotene isomers in oven dried diced carrots. In the current study flour was stored at ambient temperature hence, the carotene loss might have been due to oxidation rather than isomerization.

3.2.2. Effect of packaging and storage on total carotenoid loss of OFSP flour

Significant differences ($P \leq 0.001$) in total carotenoid content were observed among the packaging materials in both storage conditions (Figure 3-2). The highest carotenoid loss was found in kraft paper, followed by HDPE, while AFL was the least. However in most cases, the carotenoid content of flour in HDPE was comparable to AFL during the entire storage period regardless of the storage environment.

Overall, during the four mo storage period total carotenoid content of flour in kraft paper was 7.84±0.02mg/100 g DW for Vita flour and 6.24±0.13 mg/100 g DW for Kabode flour under

light exposure (Figure 3-2). Under dark conditions, the carotenoid contents for Vita and Kabode flours in kraft paper were 7.98±0.08 mg/100 g DW and 6.4±0.10 mg/100 g DW, respectively (Figure 3-2). Thus, the total carotenoid loss for kraft paper under light condition was 54.43% (Vita flour) and 59.33% (Kabode flour). The total carotenoid loss under dark condition were 53.58% for Vita flour and 58.27% for Kabode flour. Emenhiser et al. (1999) attributed high β-carotene loss to oxygen permeability in sweetpotato chips stored under ambient conditions. The current results confirm oxygen as the main factor causing carotenoid degradation of flour during storage. Non-vacuum sealing of flour in Kraft paper led to high oxygen transmissivity into the package causing carotenoid oxidation, hence, losses.

When flour was packed in HDPE, the carotenoid content ranged from 13±0.12 mg/100 g DW to 10.7±0.32 mg/100 g DW after 4 mo under storage exposed to light while under dark storage it ranged from 14.97±0.53 to 8.9±0.03 mg/100 g DW (Figure 3-2). This translates to 49.08% and 41.46% carotenoid loss for Kabode and Vita flour, respectively. In contrast, the percent carotenoid loss for flour in HDPE under dark conditions was 39.24% (Vita flour) and 40.64% (Kabode flour). No significant differences ($P > 0.05$) in carotenoid content were observed between the two storage environments. This further confirms that oxygen, and not light, was the main factor causing carotenoid degradation (Emenhiser et al., 1999). Cinar (2004) reported photoisomerization as having insignificant effect as opposed to oxidation. Additionally, the vacuum sealing of flour in HDPE paper compacted the pack, so it was only the small outer layer that was exposed to light while the rest of the inner layer was not affected.

AFL paper had the least carotenoid loss both under light and dark conditions. The carotenoid loss of flour in AFL pouches ranged from 41.5% to 35.03% under conditions exposed to light, while under dark conditions it ranged from 30.4% to 29.88%. The reason for low

carotenoid loss of flours in AFL might be due to the opaque nature of AFL as well as vacuum sealing, which might have prevented carotenoid degradation through photoisomerization and oxidation, respectively. Beyond week 8, carotenoid loss was drastic. This might be due to accumulation of oxygen in the pouch, hence suggesting carotenoid loss through oxidation. Any packaging material has an oxygen transmission rate at a particular temperature and humidity. For instance the oxygen transmission rate of AFL at 23°C , 0% RH and 1 atm is 0.01 cc in^2/ day while water vapor transmission rate is 0.005 g in^2/day at 100% RH, 23°C and 1 atm (Emenhiser et al., 1999). During the storage time, average temperature and RH were 23°C and 24%, respectively.

Although not statistically significant ($P > 0.05$), flour from the Kabode genotype was more susceptible to carotenoid degradation under dark and light storage conditions. Similarly, there was no significant difference ($P > 0.05$) in carotenoid content between flours stored in dark and light conditions. The findings confirm oxygen has greater impact on carotenoid degradation through oxidation as previously reported by Bechoff et al. (2010a) and Emenhiser et al. (1999).

According to Figure 3-3, it is clear that the greatest carotenoid degradation was observed during the first 3 weeks of storage both under light and dark conditions. From week 4, carotenoid degradation was occurring at a decreasing rate up to week 16 in all the samples. The results indicate that the first 3 weeks are critical in minimizing carotenoid loss during storage. Efforts to prevent carotenoid degradation during storage should target the first 3 weeks and any handling activities prior to storage. It can also be concluded from the results that HDPE and AFL are appropriate packaging for OFSP flours for high carotenoid retention. Where possible, storing of OFSP fours in HDPE and AFL pouches should be vacuum sealed to achieve maximum carotenoid retention. The biggest challenge of using AFL pouches to package OFSP lies in the high cost of the material and might, therefore, limit its use. For instance in Nairobi, Kenya HDPE costs KES 1200/100

pieces while AFL costs 2300/100 pieces. This explains why AFL is used to package high value crops like coffee. Perhaps governments should subsidize AFL pouches for packaging of OFSP flour.

3.2.3. Color

Slight, but significant ($P < 0.05$), decrease in a* and b* values were observed in all of the samples stored under light and dark conditions (Figures 3-3 and 3-4). When OFSP flour was packed in Kraft paper, significant decreases in color values were observed during the entire storage period under both light and dark conditions. It is possible that light permeated into the package since it was brown in color. Non vacuum sealing of Kraft paper also might have led to light permeation into the pouches hence loss of color. Flours in HDPE also exhibited non-significant decrease in a* and b* values under both conditions. Flours in AFL exhibited almost constant values of a* and b* values when exposed both to light and dark storage conditions and results were not significant. However, a slight increase in L* values of flours was observed when flours were packed in Kraft paper and HDPE under light and dark storage conditions indicating increase in lightness of flour. The L* values of flours in AFL were almost constant during the entire storage period implying that it provided good barrier against light. It is clear from the results that the a* values for Vita and Kabode flours were comparable. The b* and L* values of Kabode flour were slightly higher than Vita flour regardless of the packaging material. The high L* values of Kabode flours indicated that it was lighter than Vita flours. Generally, the loss of flour color experienced in this study was due to exposure to light during drying of OFSP chips in the solar dryer. Drying of OFSP chips in the solar dryer for 4 d exposed the product long enough to light to contribute to color loss. Effects of the light exposure continued during flour storage.

3.2.4. Correlation between color values and β-carotene of flour

Some studies have linked the a* and b* values to β-carotene content in sweetpotatoes (Takahata et.al., 1993; Bengtsson et al., 2008). The authors reported the a* values as having the highest correlation to β-carotene content in OFSP fresh roots with correlation coefficients of 0.9 and 0.96, respectively. However, in our study the a* value did not strongly correlate with the β-carotene content. The correlation coefficient for light (r = 0.66) and dark (r = 0.68) storage condition ($P < 0.001$) indicate a positive but slightly weak relationship between a* values and β-carotene content of the flours. During progressive storage of OFSP flours carotenoid loss was so rapid while color loss was gradual to constant values resulting in the weak correlation between a* value and β-carotene content. The gradual loss in color of stored flour is related to the packaging that prevented total access by light. Therefore, color can be used as an indicator of β-carotene content in fresh roots and exposed flours only. Where carotenoid rich food is processed and stored for a period of time, β-carotene content and color values can be affected by many factors. Gross (1991) suggested complexing of carotenoids with proteins, caramelization, phenolic action and enzymatic browning as the main factors responsible for color change in carotenoid rich processed products. In this study, enzymatic browning during preparation and drying of OFSP chips might influence color of flour after milling. Moreover, in this study flours were processed from unpeeled roots so the sweetpotato skin might have also affected flour color.

3.2.5. Water activity

The water activity of flours stored under conditions exposed to light increased from 0.432 to 0.601 during the 4 mo storage period (Figure 3-5). A similar trend in water activity of flour during storage was observed when flours were stored under dark conditions (Figure 3-5).

Significant differences ($P \leq 0.001$) in water activity were observed among the treatments from week 4 to week 16 under both conditions although interaction between genotype, packaging materials and storage environment was not significant ($P \geq 0.05$). Flours in Kraft paper bags had the highest water activity, indicating migration of moisture from the environment into the package. It was also observed that the water activity of flours in HDPE and AFL increased gradually during the entire storage period. This implies that HDPE and AFL pouches provided a good barrier to moisture transmission during the storage of flour. The current findings are in agreement with findings by Sra et al. (2014) and Swain et al. (2013). Sra et al. (2014) reported a significant increase in water activity of stored, diced carrot slices from 0.365 to 0.432 during 6 mo of storage. According to Swain et al. (2013), aluminum foil and HDPE had a non-significant effect on water activity for both stored red and yellow capsicum. Therefore, HDPE and AFL are appropriate packaging materials for OFSP flour.

Correlations between water activity and β-carotene of stored flours were a negative but very strong relationship. The correlation coefficient between the two parameters for flour under conditions exposed to light was 0.88 while under dark conditions it was 0.89 ($P < 0.001$). The results indicate that an increase in water activity was associated with a significant decrease in β-carotene of flours during storage. Lavelli et al. (2007) reported that carotenoids are relatively stable over a water activity range of 0.31–0.57 in freeze-dried carrot. Thus, water activity can also be used as a reliable indicator in the assessment of β-carotene content in flour during storage.

Although data on sorption isotherms of stored OFSP flours was not collected, Bechoff (2010b) and Lavelli et al. (2007) have reported of the same in relation to water activity and carotenoid degradation. Bechoff (2010b) found that samples stored at $_{a}w=0.13$ showed greater

losses of β-carotene, followed by those stored at a_w = 0.30, 0.51 and 0.76. The study demonstrated that storage at high water activity (a_w =0.76) improved β-carotene retention, but such practice would not be recommended due to high probability of microbial spoilage. Bechoff (2010b) further reported a linear relationship between the β-carotene degradation rate and water activity (R_2=0.952) which agrees with the current findings. The rapid carotene degradation at low water activities might have contributed to the total carotene breakdown at high water activities in the long run.

3.2.6. Conclusions

The study has demonstrated that AFL with vacuum sealing is a suitable packaging material for storing OFSP flour with high carotenoid retention and minimal increase in product water activity. Packing of flour in AFL should be vacuum sealed for high carotenoid retention. It is also evident from the study that the association between a* value and β-carotene content of flour was positive but weak. Increased water activity of OFSP flours was strongly, but negatively, associated with β-carotene content. The low water activity of flour in AFL makes the pouch the appropriate packaging for stored OFSP flour. Future studies should focus on assessing economic analysis of AFL in packaging of OFSP flours.

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Conflict of interest statement

The authors declare that there is not conflict of interest

REFERENCES

Abidin, P. E., Dery, E., Amagloh, F. K., Asare, K., Amoaful, E. F., & Carey, E. E. (2015). Training of trainers' module for orange-fleshed sweetpotato (OFSP) utilization and processing. International Potato Center (CIP); Nutrition Department of the Ghana Health Service, Tamale (Ghana). 32pp

AOAC. (1984). Official Methods of Analysis of the Association of Official Analytical Chemists. The Association of Official Analytical Chemists, Inc. Arlington, VA.

Achir, N., Pénicaud, C., Bechoff, A., Boulanger, R., Dornier, M., & Dhuique-Mayer, C. (2014). Use of multi-response modelling to investigate mechanisms of β-carotene degradation in dried orange-fleshed sweet potato during storage: from carotenoids to aroma compounds. Journal of Food Bioprocessing Technology, 7, 1656–1669

Bechoff, A., Dufour, D., Dhuique-Mayer, C., Marouzé, C., Reynes, M. & Westby, A. (2009). Effect of hot air, solar and sun drying treatments on provitamin A retention of orange-fleshed sweet potato. Journal of Food Engineering, 92, 164-171

Bechoff, A., Westby, A., Owori, C., Menya, G., Dhuique-Mayer, C., Dufour D. & Tomlins, K. (2010a). Effect of drying and storage on the degradation of total carotenoids in orange-fleshed sweetpotato cultivars. Journal of the Science of Food and Agriculture, 90, 622-629

Bechoff, A. (2010b). Investigating carotenoid losses after drying and storage of orange-fleshed sweet potato. Natural Resources Institute, University of Greenwich

Bengtsson, A., Namutebi, A., Almingera, M. L., & Svanberg, U. (2008). Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. Journal of Food Composition and Analysis, 21, 134-143

Chandler, L. A., & Schwartz, S. J. (1988). Isomerization and losses of trans-β-carotene in sweet potatoes as affected by processing treatments. Journal of Agricultural and Food Chemistry, 36, 129–133

Cinar, I. (2004). Carotenoid pigment loss of freeze-dried plant samples under different storage conditions. Lebensm Wiss Technology, 37, 363–367

Doering, W., Von, E., Sotiriou-Leventis, C., & Roth, W. R. (1995). Thermal interconversions among 15-cis, 13-cis, and all trans-β-carotene: kinetics, Arrhenius parameters, thermochemistry, and potential relevance to anticarcinogenicity of all-trans-β-carotene. Journal of American Chemical Society 117, 2747–2757

Emenhiser, C., Watkins R. H., Simunovic, N, Solomons, N., Bulux, J., & Barrows, J. (1999). Packaging preservation of β-carotene in sweetpotato flakes using flexible film and an oxygen absorber. Journal of Food Quality, 22, 63–73

Gross, J. (1991). Pigments in Vegetables, Chlorophylls and Carotenoids. Van Nostrand Reinhold, New York, USA

Hagenimana, V. & Owori, C. (1996). Feasibility, acceptability, and production costs of sweetpotato-based products in Uganda. Program Report 95-96. International Potato Centre (CIP) http://www.cipotato.org/Market/PgmRprts/pr95-96/program6/prog68.htm#Methods

Hagenimana, V., Carey, E., Gichuki, S. T., Oyunga, M. A. & Imungi, J. K. (1999). Carotenoid contents in fresh, dried and processed sweetpotato products. Journal of Ecology in Food and Nutrition, 37, 455-473

Hall, A., Bockett, G. & Nahdy, S. (1998). Sweetpotato Postharvest Systems in Uganda: Strategies, Constraints, and Potentials. Social Science Department International Potato Center, Lima, Peru.

Hiranvarachat, B., Suvarnakuta, P., & Devahastin, S., (2008). Isomerisation kinetics and antioxidant activities of β-carotene in carrots undergoing different drying techniques and conditions. Journal of Food Chemistry, 107, 1538–1546

Lavelli, V., Zanoni, B., & Zaniboni, A. (2007). Effect of water activity on carotenoid degradation in dehydrated carrots. Journal of Food Chemistry, 104, 1705–1711

Muzhingi, T.; Yeum, K.J.; Russell, R.M.; Johnson, E.J.; Qin, J.; Tang, G. (2008). Determination of carotenoids in Yellow Maize, the effects of saponification and food preparations. International Journal of Vitamin and Nutrition Research, 78, 112–120

Nungo, R. A., Ndolo, P. J. & Hagenemana, V. (2000). Promoting sweet potato processing and utilization: Experience in Western Kenya. African Potato Association Conference Proceedings, 5, 481-482

Ray, R. C. & Tomlins, K. L. (2010). Sweet Potato: Post Harvest Aspects in Food, Feed and Industry. Nova Science Publishers, Inc., New York.

Rodriguez-Amaya, D. B., Nutti, M. R. & Viana De Carvalho, J. L. (2011) Carotenoids of sweet potato, cassava, and maize and their use in bread and flour fortification and their Fortification in Health and Disease Prevention. (V.R.P.R. Patel, ed.). Academic Press, San Diego.

Sra, S. K., Sandhu, K. S. & Ahluwalia, P. (2014). Effect of treatments and packaging on the quality of dried carrot slices during storage. Journal of Food Science and Technology, 51(4), 645–654

Swain, S. Samuel D. V. K & Abhijit K. A. R. (2013). Effect of packaging materials on quality characteristics of osmotically pretreated microwave assisted dried sweet pepper (*Capsicum*

annum L.). Journal of Food Processing and Technology, 4: 264. doi:10.4172/2157-7110.1000264

Takahata, Y., Nona, T. & Nagata, T. (1993). HPLC determination of β-carotene content of sweetpotato cultivars and its relationship with color values. Japanese Journal of Breeding, 43, 421-427

Tewe, O. O. Ojeniyi F. E. & Abu O. A. (2003). Sweetpotato production, utilization, and marketing in Nigeria. Social Sciences Department, International Potato Center (CIP), Lima, Peru.

Teow, C. C., Truong, V., Mcfeeters, R. F., Thompson, R. L., Pecota, K. V. & Yencho, G. C. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colors. Journal of Food Chemistry, 103, 829–838

van Hal, M. (2000). Quality of sweetpotato flour during processing and storage. Journal of Food Review International, 16, 1-37

Woolfe, J. A. (1992). Post harvest procedures: Sweet Potato an Untapped Food Source. Cambridge, UK: Cambridge University Press.

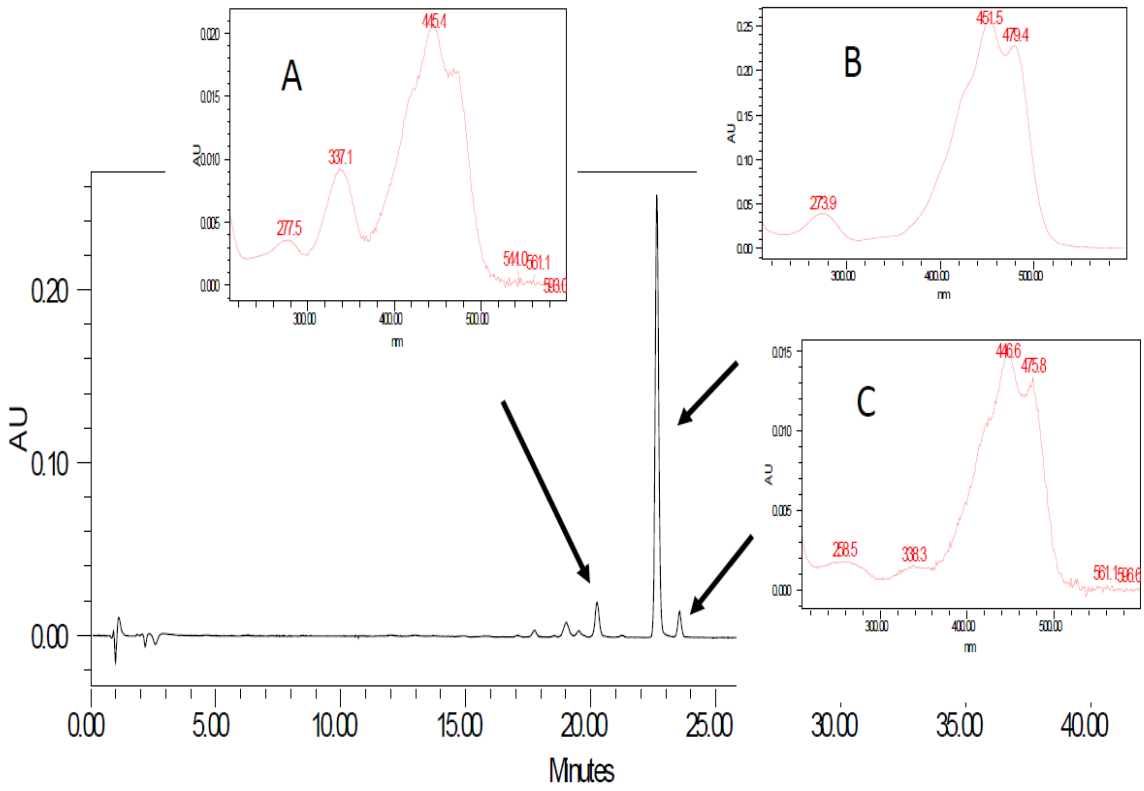

Figure 3-1. Chromatogram peaks of 13-cis β-carotene (A), All trans β-carotene (B), and 9-cis β-carotene (C).

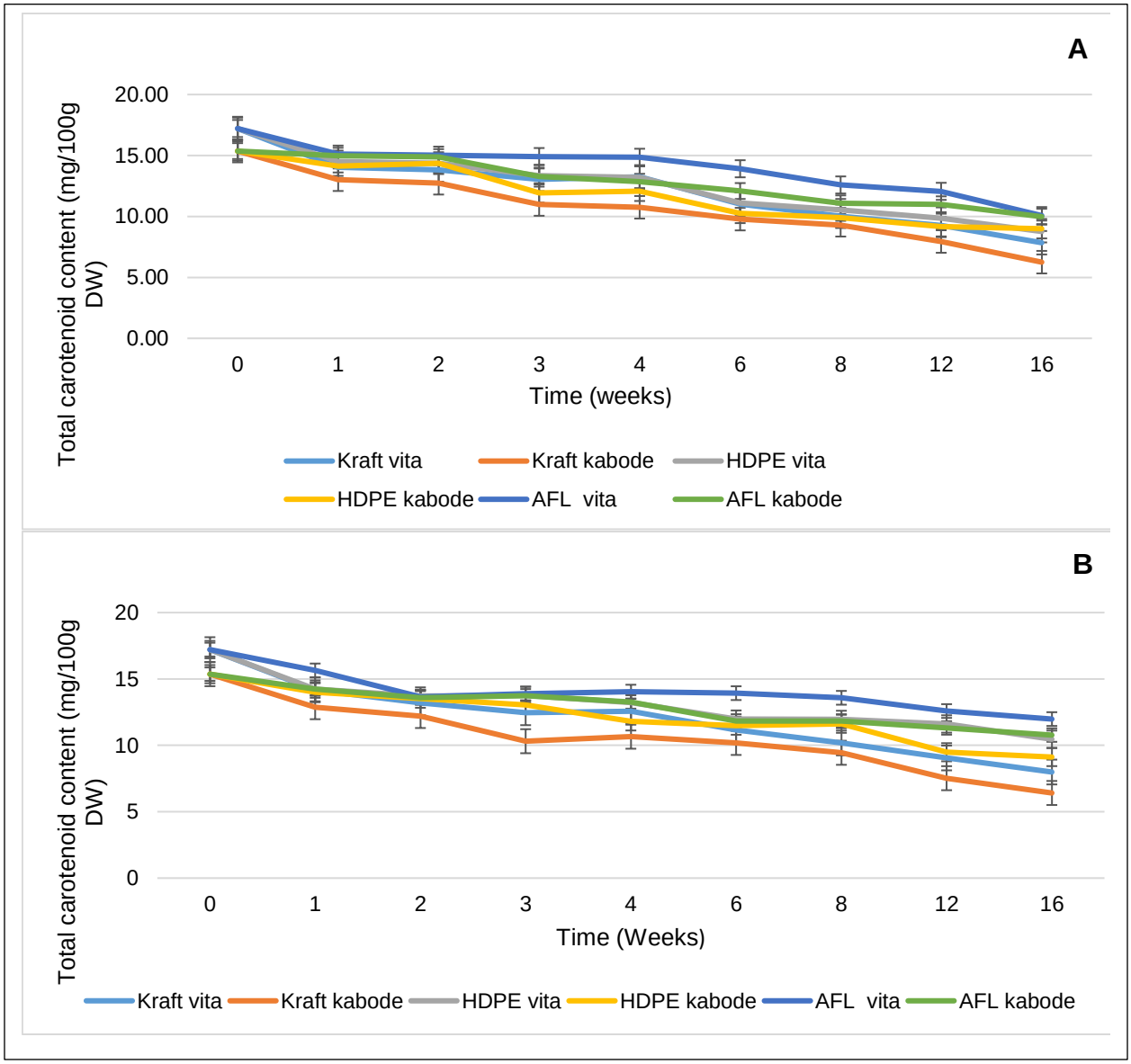

Figure 3.2 Total carotenoid content of OFSP flour stored under light condition (Panel A). Carotenoid content of OFSP flour stored under dark condition (Panel B).

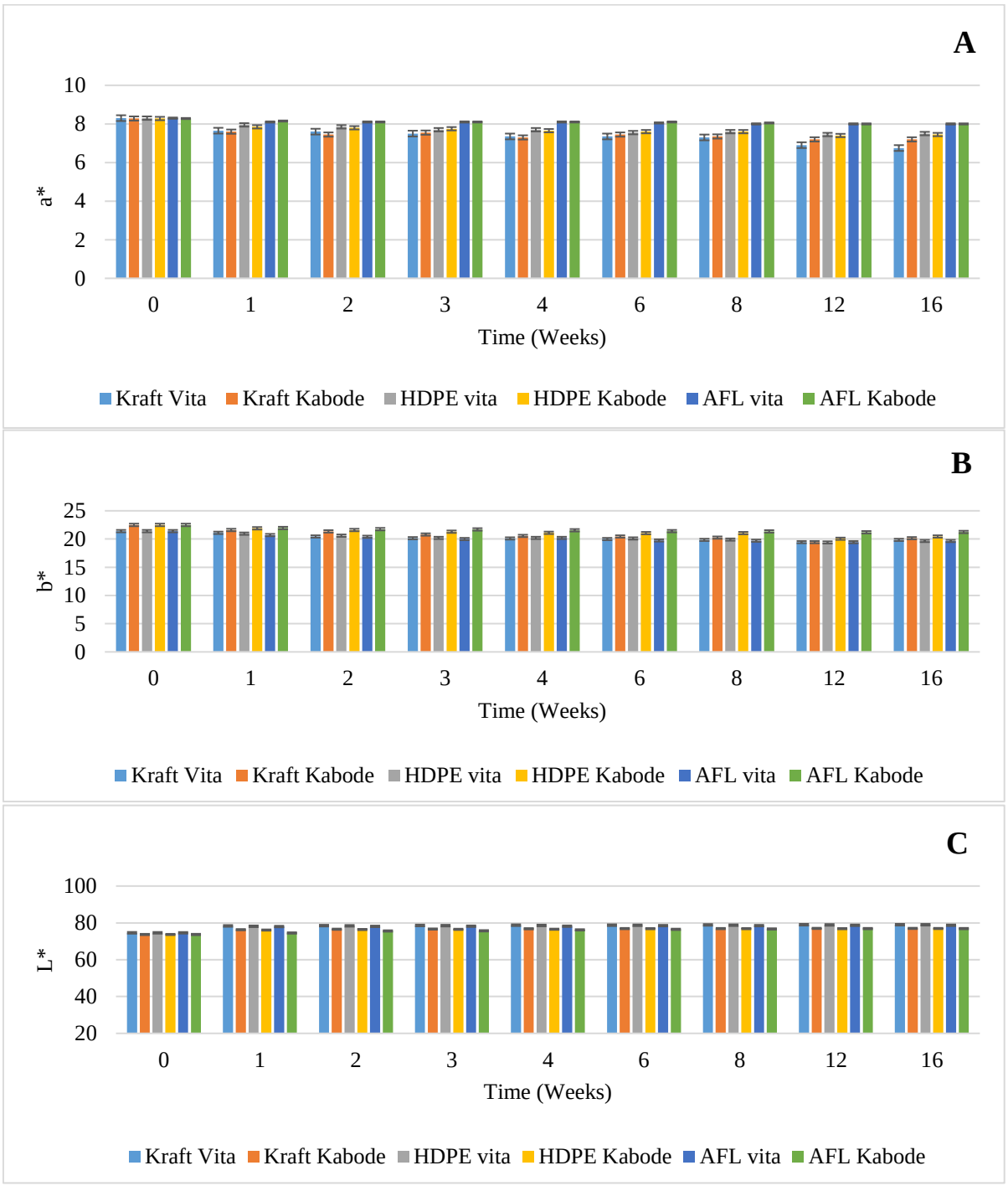

Figure 3-3. The a* (Panel A), b* (Panel B) and L* (Panel C) of flour stored under light condition

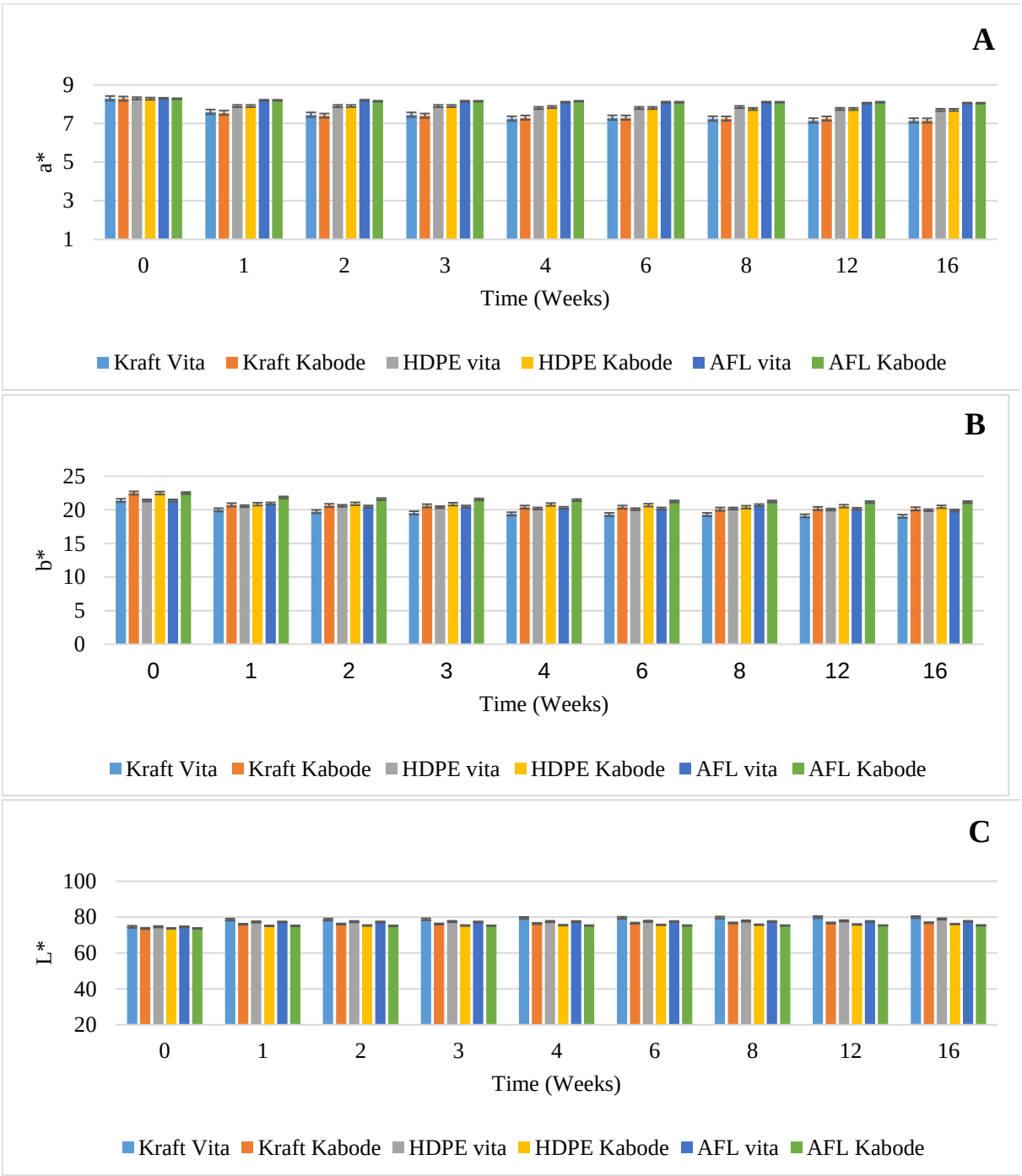

Figure 3-4. The a*, (Panel A), b* (Panel B) and L* (Panel C) of flour stored under dark condition

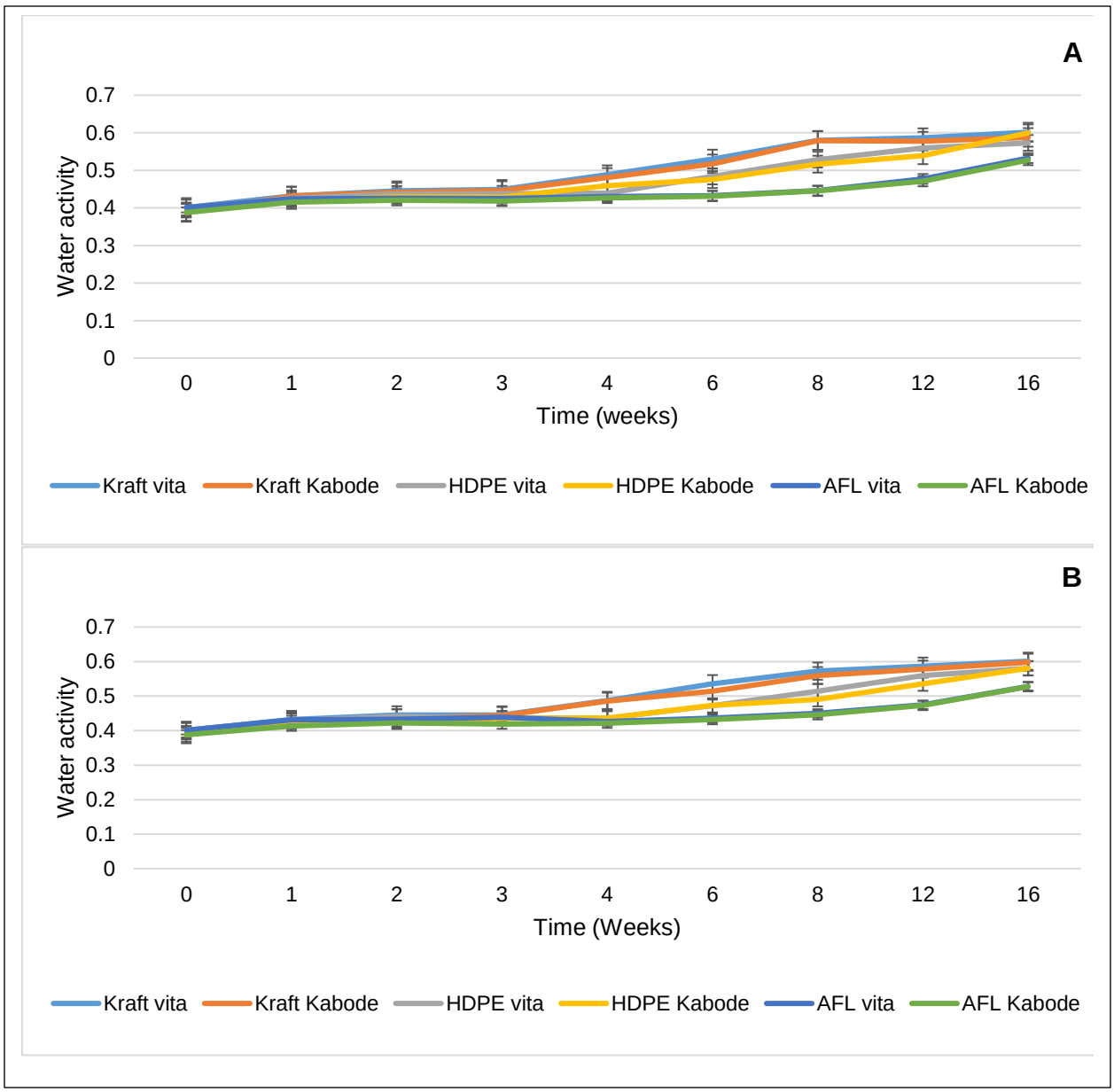

Figure 3-5. Water activity of OFSP flour stored and light condition (Panel A) and dark condition (Panel B).

CHAPTER 4.

EFFECT OF PROCESSING AND OIL TYPE ON CAROTENE BIOACCESSIBILITY IN TRADITIONAL FOODS PREPARED WITH FLOUR AND PUREE FROM ORANGE-FLESHED SWEETPOTATOES

Effect of Processing and Oil Type on Carotene Bioaccessibility in Traditional Foods Prepared with Flour and Puree From Orange-Fleshed Sweetpotatoes

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4.0. Introduction

Sweetpotato (*Ipomea batatas* Lam) is the fourth most important staple crop in sub-Saharan Africa (FAOSTAT, 2004). It is rich source of energy, fiber, minerals and vitamins. Orange-Fleshed sweetpotato (OFSP) contains β-carotene, a precursor for vitamin A (VA) in the body. In sub-Saharan Africa traditional methods of sweetpotato preparation include boiling, steaming, roasting, and drying (Hall, 1998). Dried OFSP products like chips and flour are the common ingredients for food preparations at the household level (Hall, 1998). In the recent past, the use of OFSP puree has become important due to its high carotene retention during storage as compared to flour and dried chips. Utilization of OFSP puree in bread formulation and porridge has been adopted in Kenya.

Although OFSP contains significant amounts of β-carotene, not all of the available quantity is retained after processing. Bechoff et al. (2011) reported retention of all-trans-β carotene in porridges and *chapatis* supplemented with 30% OFSP flour by 69-93% and 70-97%, respectively. Significant degradation in β-carotene content of deep fried OFSP products have been reported (Kidmose, 2006). The carotene losses of the deep fried products were mostly due to longer frying times at high temperatures causing transfer of carotenes into the oil, as they are oil-soluble. These studies have demonstrated carotenes loss during processing and storage of OFSP products. However, there is need to investigate carotene retention of various OFSP products as affected by processing and sweetpotato genotype. In addition, information on β-carotene bioaccessibility of OFSP traditional products is limited. Carotenoids bioaccessibility is defined as the fraction of carotenoids transferred by food to mixed micelles (small aggregates of mixed lipids and bile acids suspended within the ingesta), therefore becoming accessible for subsequent uptake by intestinal mucosa (Burri, 2011). It is important to have high bioacessibility

of β-carotene in OFSP in order to fully have health-benefits from the nutrient. Carotenoid bioaccessibility is affected by a number of factors such as carotenoid structure, processing, dietary fat and oil. Among the different types of carotenoids, lutein is reported to be more bioaccessible due to the fact that lutein is an oxycarotenoid and therefore more hydrophilic than hydrocarbon carotenoids (Garret et al., 1999). High temperature processing of carotene-rich foods disrupts plant cell walls and organelle membranes, facilitating greater access of digestive enzymes to substrates and release of carotenoids for integration into mixed micelles resulting in high bioaccessibility (Hendren, 2002). Bioaccessibility of β-carotene increased from 0 to 20% with addition of fat/oil to digestion (Pugliese et al., 2013; Ekase et al., 2012). Similarly, Failla et al. (2008) observed an increase in carotenes as a result of addition of triglyceride to carotenoids rich salad.

The effect of oil type on *in vitro* carotene bioaccessibility of OFSP products has not been fully explored. Therefore, the current study focused on assessing the bioaccessibility of all-trans β- carotene from OFSP products for improved VA intake and consequently contribute toward eradicating VA deficiency in Africa. The effect of oil type on β-carotene bioaccessibility was also evaluated in this study. Information generated by the study is important to determine processing methods and oil type with optimal carotene delivery after digestion for probable intestinal absorption.

4.1. Materials and Methods

4.1.1. Chemicals and standards

Reagents for chemical analysis (ethanol, methanol, tetrahydrofuran (THF), hexane, butylated hydroxytoluene, acetone, and methyl tert-butyl ether), β-carotene standards (all-trans β-carotene, 13-cis and 9-cis β-carotene isomers) and enzymes (α amylase, pepsin and pancreatin) for *in vitro* digestibility studies were purchased from Sigma Aldrich (St. Gallen, Switzerland). All local food ingredients and materials were sourced in Kenya.

4.1.2. Sweetpotato genotypes and preparation of dried chips and flours

Two OFSP genotypes namely Vita and Kabode were bought from farmers in Oyugisi, Kenya. After harvest the roots were washed with water to remove sand and dirt and immediately processed into flour and puree. For flours, sweetpotato roots were cut into 5 mm slices using a chipper and then dried in a solar dryer to a moisture content of 10%. After drying, the sweetpotato slices were milled into flour, packed in brown sack bags lined with polyethylene paper on both sides and stored at -20^{o}C until used in porridge and *chapati* preparations. For sweetpotato puree, sweetpotato roots were cleaned, trimmed and boiled unpeeled at 100^{o}C for 1 h. After cooling to room temperature, the cooked roots were pureed using a hammer mill, vacuum packed and stored at -20^{o} C until usage in porridge and *chapati* making.

4.1.3. Porridge preparation

Hundred gram of 50% OFSP flour/puree and 50% maize flour was weighed and mixed with 100 mL cold water to form a slurry. The slurry was then added to hot water while stirring to

form a paste and allowed to cook for 5 min. Hundred percent maize flour porridge was prepared as control. Cooked porridge was allowed to cool, packed in ziplock bags and stored at -80^{o}C until analysis.

4.1.4. *Chapati* preparation and effect of oil type study

Hundred grams of 50% OFSP flour/puree and 50% wheat flour blend were weighed into a mixing bowl. A pinch of salt, a tablespoon of pure sunflower oil, and water were added to form a dough. The dough was cut into small portions and then rolled to form round flat doughs. The flat doughs were roasted in a pan containing 2 tablespoons of oil at 175^{o}C for 2 min. Hundred percent wheat flour *chapati* was prepared as control. Cooled *chapatis* were packed in ziplock bags and stored in -80^{o}C until further tests were conducted.

Three oil types, namely sunflower oil, margarine, and beef fat were evaluated. The choice of the oils was based on the degree of saturation and their local availability. According to our free fatty acid analysis with gas chromatography, the composition of the oils were as follows: sunflower oil contained mostly polyunsaturated (59%), with 30% monounsaturated and 11% saturated fatty acids; margarine is hydrogenated oil, behaves more like saturated fat, and is comprised of 46% saturated, 43% monounsaturated and 11% polyunsaturated fatty acids; beef fat comprised saturated (49%), monounsaturated (42%) and polyunsaturated (9%) fatty acids. Pure sunflower oil, margarine or beef fat were added to OFSP supplemented *chapatis*. The amount of oil added was 10% (w/w) of *chapati* formulation. OFSP puree and flour without oil were the controls for the experiment. The *chapatis* were prepared and stored as explained above.

4.1.5. *In vitro* digestion

The *in vitro* digestion of porridge and *chapati* was based on the method reported by Failla et al. (2009) with some modifications. Ten-gram sample was mixed with 6 mL of oral phase (pH 6.9) buffer containing 3015 units of α-amylase enzyme and incubated in a shaking water bath at 37^{o}C for 10 min. The gastric phase was initiated by adding 30 mL 0.9% sodium chloride solution and adjusting pH to 3.5 with 1.0 N HCL. Two milliliters of 10 mg/mL pepsin enzyme was added and pH adjusted to 2.5 and final volume adjusted to 40 mL with 0.9% saline solution. The sample was incubated again at 37^{o}C for 1 h in a shaking water bath. To initiate the intestinal phase, pH was adjusted to 5.0 with 1.0 N NaOH. Two milliliters of pancreatin enzyme (20 mg/mL) and 3 mL of bile extract (30 mg/mL) were added to the sample and pH adjusted again to 6.5 with 1.0 N NaOH. This was followed by incubating the sample at 37^{o}C for 2 h. At each stage of incubation the sample was blanketed with nitrogen to prevent oxidation. After intestinal phase of digestion, about 15 mL of the digesta was transferred into a clean test tube, blanketed with nitrogen and kept in a -80^{o}C freezer awaiting carotenoid analysis. The remaining material was centrifuged at 4000 x g, 4^{o}C for 1 h. The aqueous micellar fraction was filtered through micro filter (0.45 mm), blanketed with nitrogen and kept at -80°C until carotenoid analysis. The *in vitro* digestion experiment was done in triplicate for each prepared product.

4.1 6. Carotenoid extraction and analysis

Porridge and *chapati*: Triplicate samples (about 1 g) of OFSP porridge and *chapati* were extracted three times with hexane prior to saponification with 120 µL of 80% KOH and incubating at 85^{o}C for 10 minutes. The extract was evaporated to complete dryness under a

stream of nitrogen and reconstituted with appropriate volumes of methanol:THF solution (85:15 v/v) according to extract concentration.

Digestive fractions: Intestinal digesta and aqueous micellar fractions were extracted three times with 1:3 acetone:hexane with 0.1% w/v butylated hydroxytoluene, dried using nitrogen evaporator (Organomation Associates, Berlin, MA) and then reconstituted with methanol:THF solution (85:15 v/v). The reconstitution volumes for intestinal digesta and filtered micellar fractions were 1000 µL and 150 µL respectively.

All extraction procedures were done under yellow light to minimize carotenoid degradation. Carotenoids were analyzed by reversed phase HPLC equipped with auto sampler injector, degasser, pump and Waters 9562-UV-visible photodiode array detector operating at 450 nm (Waters Corporation, Milford, MA). Separations were carried out on a 3-µm, 150 x 3.0 mm, Semibore column (YMC, Wilmington, NC). The mobile phase consisted of methanol:methyl tert-butyl ether:water (85:12:3, v/v/v), plus 1.5% ammonium acetate(w/v) (phase A), methanol:methyl tert-butyl ether:water (8:90:2, v/v/v), plus 1% ammonium acetate(w/v) (phase B). The gradient procedure was at a flow rate of 0.4 mL/min and injection volume of the sample was 30 µL. The gradient program was as follow: 100% solvent A for 2 min, followed by 9 min 90% solvent A and 10% solvent B, then 12 min 45% solvent A and 55% solvent B, a 15 min hold at 5% solvent A and 95% solvent B, then a 2 min gradient back to 100% solvent A. Standard curves of pure all-trans β-carotene, 13-cis β-carotene and 9-cis β-carotene were used to quantify the carotenoids.

4.1.7. Moisture content determination

Porridge and *chapati* samples were analyzed for moisture content at the same time as for carotenoid analysis. Determinations were made by drying triplicate 5-g samples in an oven (Gallenkamp, Leicestershire, UK) at 105^{o}C to a constant weight (minimum 24 h) (AOAC, 1984).

4.1.8. Statistical analysis

All experiments were performed with three replications. The statistical analysis of collected data was performed by ANOVA using Genstat version 6.0 to determine differences among the treatments. Significant differences among treatments was obtained by Tukey's HSD multiple rank test at $P < 0.05$.

4.2. Results and discussion

4.2.1. β-carotene content of OFSP products

The HPLC chromatogram of carotenoids in OFSP supplemented porridge and *chapati* is shown in Figure 4-1. Identification of carotenoids was achieved by retention time and absorption spectra collected between 250 and 550 nm with those of authentic standards. From the method developed in the study, 13-cis β-carotene, all-trans β-carotene, and 9-cis β-carotene were identified by peak retention times occurring at 21 min, 23 min and 24 min, respectively. Quantification of carotenoids was achieved using a calibration curve with the correlation coefficient of $\geq$ 0.998. Total β-carotene content was expressed as summation of the three carotenes. As shown in Figure 4-1, all-trans β-carotene and its isomers 13-cis and 9-cis β-carotene were the main carotenes found in the OFSP products. It is worthwhile to mention that the control products, maize flour porridge

and wheat flour *chapati* did not contain any detectable level of provitamin A carotenes, hence were not included in subsequent experiments.

4.2.2. All-trans β-carotene retention during processing

The carotene contents of the products before cooking is shown in Table 4-1. Results of carotene analysis of the products expressed in μg/g fresh weight (FW) and μg/g dry weight (DW) are presented in Tables 4-2 & 4-3 respectively. It is clear that all-trans β-carotene was the predominant carotene in all the products accounting for >70% of total carotene. This was followed by 13-cis β-carotene while 9-cis β-carotene isomer was the least. Significant differences were observed among the products in terms of all-trans β-carotene content ($P < 0.05$) (Table 4-2). Between OFSP flour and puree, products made from flour had more all-trans β-carotene content than those made with puree. For instance, the all-trans β-carotene contents in vita flour *chapati* and kabode flour *chapati* were 37.91 μg/g FW and 36.22 μg/g FW respectively while vita puree *chapati* and kabode puree *chapati* had 17.46 μg/g FW and 11.64 μg/g FW respectively. A similar trend was observed when the porridges were supplemented with either OFSP puree or flour. The total β-carotene contents of vita flour and kabode flour porridge were significantly higher than vita puree and kabode puree porridge ($P < 0.05$) (Table 4-3) but results were not significant. The result might be related to high moisture content of porridge and puree based-products compared to flour although puree-based *chapatis* had low moisture content probably due to high roasting temperature. With reference to the moisture content of the products, the dry weight basis (Table 4-3) gave a different trend whereby the carotene content of porridge was higher than *chapati*. The results imply that considering the fresh weight basis, we benefit more of the nutrient in products with low moisture content. In the same line, high

moisture content foods are nutrient dense and more beneficial nutritionally on dry weight basis. Thus, we might obtain more β-carotene from OFSP *chapati* and porridge on fresh weight and dry weigh basis respectively. Ideally we consume porridge on fresh weight basis so for the same amount of β-carotene, more of the porridge will have to be consumed (as is) compared to *chapati*. The carotene content of puree-based products did not significantly improve on dry weight basis compared to flour-based products. This was due to high moisture content of puree which might have contributed to excessive dilution of total carotene when mixed with non-OFSP flour. It is seen from the results that there were no significance differences ($P \geq 0.05$) in all-trans β-carotene content among the products containing either OFSP puree or flour (Tables 4-2 & 4-3). Overall, on fresh weight basis, OFSP supplemented *chapatis* had high all-trans β-carotene content compared to porridge. The findings are in accordance with Bechoff et al. (2011), who reported higher all-trans β-carotene content from *chapatis* than porridge supplemented with 30% OFSP flour. The relatively low all-trans β-carotene of porridges might be related to its degradation associated with longer cooking time. The average cooking time of porridge was 5 min while *chapati* was 2 min, thus, allowing more carotene degradation in porridge. The low moisture content of *chapatis* might have also contributed to higher carotene content as compared to porridge with higher moisture content on wet weight basis.

Beta carotene is susceptible to degradation during processing at temperatures above 37^{o}C. In this study, apparent all-trans β-carotene retention of the products was determined by comparing carotene content in finished products (Table 4-2) to the initial content in OFSP composites flours and/or puree in product formulation (Table 4-1). All-trans β-carotene retention of *chapatis* ranged from 76% (kabode puree *chapati*) to 82.33% (vita flour *chapati*) while among the porridges it ranged from 65% (kabode flour porridge) to 71% (vita flour porridge) ($P < 0.05$).

The findings are comparable to carotene true retention in OFSP *chapatis* and porridges reported by Bechoff et al. (2011). Kean et al. (2008) reported carotene retention of 52% (yellow maize porridge) and 75% (yellow maize bread). The authors attributed isomerization of carotene during thermal processing as the main cause for decrease in carotene content. Bechoff et al. (2011) explained the lower carotene retention in porridge was possibly due to increased heat damage to carotenoids caused by their greater dispersion in boiling water. Increase in 13-cis and 9-cis β-carotene isomers as a result of high temperature processing were also reported (Bechoff et al., 2011; Chandler, 1988). However, the effect of β-carotene isomerization as a result of high temperature processing was not observed in the current study. Differences in OFSP genotype, ingredient interactions and differences in processing conditions may partially explain, the observed differences. Apart from isomerization of carotenes during thermal processing, Bechoff et al. (2018), explained physical losses such as leaching of carotenes into water experienced at an initial step of processing as a factor responsible for carotene degradation in processed products. It is important to mention that the carotene retentions reported in this study were calculated based on fresh weight of the products, since they are consumed fresh. A different scenario would be observed on dry weight basis as shown in Table 4-3.

4.2.3. *In Vitro* β-carotene bioaccessibility in OFSP products

The effect of processing on digestive stability and micellarization efficiency of all-trans β-carotene was evaluated on digesta and filtered micellar of OFSP porridge and *chapatis* respectively. Digestive stability indicates the percentage of the carotenoids recovered in the digesta following simulated digestion of OFSP products. It was calculated as amount of carotenoids in the digesta expressed as a percentage of the amount of carotenoids in the

undigested food product. The percentage of carotenoids transferred from the digesta to the filtered aqueous fraction is defined as the micellarization efficiency and is used as a measure of relative bioaccessibility. Micellarization efficiency was calculated as the amount of carotenoids in the aqueous micellar fraction expressed as a percentage of the amount of carotenoids in the digesta. As shown in Figure 4-2, percent digestive stability of all-trans β-carotene of porridge ranged from 52% to 72.9% whereas in *chapatis* it ranged from 69% to 87%, and the results were not dependent on sweetpotato genotype. The current findings of carotene recovery after simulated digestion are slightly lower than the recovery values of >100% reported by Kean et al. (2008). Despite the differences with published literature, the carotene recovery obtained in this study was >50% indicating good stability of carotene in the products during simulated digestion. As shown in Table 4-2, puree-based products had low carotene content compared to flour-based products, a similar trend was observed on carotene recovery after digestion. It is documented that in the food matrix, carotenes are found complexed to proteins in chromoplasts forming a proteinaceous complex (Garrett et al., 2000). Such complex microstructure has major implications on the release of carotenes during processing, simulated digestion, extraction and analysis. Processing methods that cause more disruption of cell matrix result in high carotene recovery after digestion. This explains in part the high digestive recovery of OFSP flour-based porridge and *chapatis*. The maceration of OFSP roots during chipping step as well as milling of dried chips into flour caused cell rupture and facilitated release of carotene from the matrix, resulting in improved carotene delivery. On the other hand, the processing step of puree did not include severe maceration of the cells such as chipping and milling resulting in low carotene recovery after simulated digestion.

The mean efficiency of micellarization for all-trans β-carotene varied between products. The all-trans β-carotene micellarization ranged from 11 to 18% for porridge and from 40 to 62% for *chapati* (Figure 4-2). With reference to the OFSP primary product, flour-based products had high micellarization efficiency compared to puree-based products. The result is similar to the findings by Bechoff et al. (2011) who reported high micellarization efficiency for OFSP flour porridge compared to boiled and mashed OFSP. Bengsston et al. (2009) reported a close relationship between damaging effect of drying on sweetpotato cell integrity and bioaccessibility. Moreover, cell disruption during chipping of roots and grinding of OFSP dried chips facilitated release of carotene from cell matrix and giving great access by enzymes during *in vitro* digestion resulting in improved bioaccessibility of flour-based products. In the same way, the kneading step of *chapati* dough facilitated release of all-trans β-carotene from the food matrix making it more accessible to digestive enzymes. Processing temperature is another factor affecting carotene bioaccessibility. Hendren (2002) explained that high temperature processing disrupts plant cell wall and organelle membrane facilitating greater access by digestive enzymes to substrate and carotene release for incorporation into mixed micelle, hence improved bioaccessibility. Thus, the high processing temperature of *chapatis* (175^{o}C) compared to porridge (100^{o}C) might have influenced high carotene bioaccessibility.

In general, the micellarization efficiency trend corresponded well to digestive recovery further confirming stability of carotene to simulated digestion. The lower levels of 13-cis and 9-cis β-carotene in the products consequently affected their digestive recoveries and apparent bioaccessibility (results not shown). This is contrary to findings by Bechoff et al. (2011) who reported improved bioaccessibility of cis isomers compared to all-trans β-carotene. It is important to mention that the current all-trans β-carotene micellarization efficiencies of OFSP

products are in line with previous studies (Thakkar & Failla., 2008; Bechoff et al., 2011; Lipkie et al., 2013 and Failla et al., 2009). The percentage of micellarized all-trans β-carotene was 16% for porridge and 73% for *chapati* (Bechoff et al., 2011). Considering the processing method, it is clear that addition of oil in *chapati* greatly improved micellarization efficiency. Lipikie at al. (2013) also observed improved bioaccessibility with addition of 10% oil in porridge prior to simulated digestion. Stir-frying of carotene rich food was reported to lead to high carotene bioaccessibility (Garrett et al., 2000: Veda et al., 2010). Authors attributed improved carotene micellarization efficiency to addition of oil, as oil can facilitate incorporation of carotene into the mixed micelle during digestion. The VA activity of OFSP supplemented porridge and *chapati* to meet nutritional requirements among different groups is well documented by Bechoff et al. (2011) and similar estimates can be applied to the products in the current study.

4.2.4. Effect of oil type on β-carotene bioaccessibility

In the present study the effect of three different oil types, including pure sunflower oil, margarine and beef fat, on *in vitro* bioaccessibility of β-carotene were evaluated and results are presented in Figure 4-3. According to Figure 4-3, the digestive stability of the products were similar to data presented in Figure 4-2 indicating that all-trans β-carotene of OFSP products is not affected by oil type. However, efficiency of transfer of all-trans β-carotene from the aqueous phase to the filtered micellar phase was significantly affected by oil type. The greatest micellarization efficiency was found in *chapati* with sunflower oil (67.8%), followed by margarine (30.0%) while beef fat (9.3%) was the least. The micellarization efficiencies of the control products were significantly lower than *chapatis* with sunflower oil and margarine but higher than *chapatis* with beef fat. Beta-carotene is a lipophilic micronutrient, hence its

bioaccessibility is greatly affected by presence of oil during digestion. Sunflower oil, a predominantly long-chain unsaturated fatty acid with low melting point is effective in dispersing and dissolving β-carotene at the digestion temperature, thus facilitating its incorporating into the mixed micelles. On the other hand, margarine and beef fat are more saturated oils characterized by high melting point, hence not as effective at dissolving β-carotene at the digestion temperature. Although margarine contained high all-trans β-carotene content (data not shown), its presence did not enhance all-trans β-carotene of *chapatis*. The high micellarization efficiencies of the control products compared to beef fat is indicative of inability of beef fat to solubilize β-carotene at the digestion temperature as most of the nutrient was trapped in its bulk structure. The result further confirms that oil type has great impact on carotene bioaccessibility. Apart from dissolving carotene and facilitating incorporation of carotene into mixed micelles, the presence of oils also induces secretion of bile and pancreatic juice in the digestive tract.

Several studies have linked presence of oil to β-carotene bioaccessibility (Pugliese et al., 2013; Ekase et al., 2012, Failla et al., 2008). Micellarization efficiency of β-carotene during small intestinal digestion was increased by lipids rich in unsaturated fatty acids: soybean oil > olive > canola > butter (Failla et al., 2014). On the contrary, polyunsaturated fatty acids reduced β-carotene bioaccessibity due to formation of peroxides of the nutrient during digestion (Nagao et al., 2013). In a separate study, Berni et al., (2014) reported a significant increase in all-trans β-carotene micellarization efficiency of fried cassava compared to boiled cassava. Bengtsson et al., (2010) linked improved micellarization efficiency to cooking style alongside oil addition. Thus, cooking style involving cell disruption and oil addition improved β-carotene bioaccessibility. However there is a limit to which dietary oils or fats can improve carotene bioaccessibility. Presence of excess oil relative to digestive enzyme during simulated digestion suppressed

carotene bioaccessibility (Nagao et al., 2013). The effects of oil type on β-carotene bioaccessibility reported by the present study is relevant *in vivo*. *Chapati* is a staple food commonly consumed in sub-Saharan Africa. The current practice of *Chapati* making involves addition of margarine or shortening as a source of dietary oil/fat. Based on the current results, margarine gave low carotene bioaccessibility. Alternatively sunflower oil with high carotene bioaccessibility could be used as source of dietary oil in *chapatis*. In Africa sunflower oil is more available at affordable price and people could take advantage of this to aid in *chapati* formulation for improved carotene bioaccessibility to fully benefit from the nutrient.

In conclusion, the current study has indicated that the type of food product and associated processes may affect carotene bioaccessibility. Processing methods with high cell disruption have higher carotene bioaccessibility. All-trans β-carotene micellarization efficiency varied between OFSP products and was higher in *chapatis* than porridge but results were not affected by sweetpotato genotype. Addition of oil in OFSP-supplemented products is critical to ensure greater carotene bioaccessibility. Long-chain unsaturated oils, like sunflower oil, have greater carotene bioaccessibility than margarine and beef fat. These results confirm that OFSP can serve as a source of bioaccessible provitamin A carotenoids. Future research needs to assess bioavailability using Caco-2 cells or a similar cell model for trans-epithelial absorption. Human studies are warranted to confirm *in vivo* the efficacy toward improving VA status in target populations. There is need for wide screening of OFSP genotypes to ensure development of genotypes with highly bioaccessible carotene.

REFERENCES

AOAC. (1984). Official Methods of Analysis of the Association of Official Analytical Chemists; The Association of Official Analytical Chemists, Inc.: Arlington, VA.

Bechoff, A., Poulaert, M., Tomlins, K., Westby, A., Menya, G., Young, S., & Dhuique-Mayer, C. (2011). Retention and bioaccessibility of β-carotene in blended foods containing orange-fleshed sweet potato flour. Journal of Agricultural and Food Chemistry, 59, 10373–10380

Bechoff, A., Tomlins, K., Chijioke, U., Ilona, P., Westby, A., & Boy, E. (2018). Physical losses could partially explain modest carotenoid retention in dried food products from biofortified cassava. PLoS ONE 13(3): e0194402. https://doi.org/10.1371/journal.pone.0194402

Bengtsson, A., Alminger, L., & Svanberg, M. (2009) In vitro bioaccessibility of β carotene from heat processed orange-fleshed sweet potato. Journal of Agricultural and Food Chemistry, 57, 9693–9698

Bengtsson, A, Brackmann, C, Enejder, A, Alminger, L & Svanberg, M. (2010). Effects of thermal processing on the in vitro bioaccessibility and microstructure of β-carotene in orange-fleshed sweet potato. Journal of Agricultural and Food Chemistry, 58, 11090–11096

Berni, P, Chitchumroonchokchai, C., Canniatti-Brazaca, G. De Moura, F., & Failla, L. (2014). Impact of genotype and cooking style on the content, retention, and bioacessibility of β-carotene in biofortified cassava (*Manihot esculenta Crantz*) conventionally bred in Brazil. Journal of Agricultural and Food Chemistry, 62, 6677−6686

Burri, B.J. (2011). Evaluating sweetpotato as an intervention food to prevent Vitamin A deficiency. Comprehensive Reviews in Food Science and Food Safety, 10, 118-130

Chandler, L.A. & Schwartz, S.J. (1988). Isomerization and losses of trans-β-carotene in sweet potatoes as affected by processing treatments. Journal of Agricultural and Food Chemistry, 36, 129-133

Ekesa, B., Poulaert, M., Davey, M.W., Kimiywe, J., Van den Bergh, I., Blomme, G. & Dhuique-Mayer, C. (2012). Bioaccessibility of provitamin A carotenoids in bananas (*Musa* spp.) and derived dishes in African countries. Journal of Food Chemistry, 133, 1471–1477

Failla, M.L., Chitchumronchokchai, C., Ferruzzi, M.G., Goltz, S.R. & Campbell, W.W. (2014). Unsaturated fatty acids promote bioaccessibility and basolateral secretion of carotenoids and α-tocopherol by Caco-2 cells. Food and Function, 5, 1101–1112

Failla, M.L., Thakkar, S.K., & Kim, J.Y. (2009). In vitro bioaccessibility of β-carotene in orange-fleshed sweet potato (*Ipomoea batatas* Lam). Journal of Agricultural and Food Chemistry, 57, 10922–10927

FAOSTAT. (2004). The state of food insecurity in the world. Rome, Italy: Food and Agriculture Organization of the United Nations

Garret D.A., Failla M.L. & Sarama R.J. (1999). Development of in vitro digestion method to assess carotenoid bioavailability from meals. Journal of Agricultural and Food Chemistry, 47, 4301-9

Garrett, D.A., Failla, M.L., & Sarama, R.J. (2000). Estimation of carotenoid bioavailability from fresh stir-fried vegetables using an in vitro digestion/Caco-2 cell culture model. Journal of Nutrition Biochemistry, 11, 574–580

Hall, A., Bockett, G., & Nahdy, S. (1998). Sweetpotato postharvest systems in Uganda: strategies, constraints, and potentials; International Potato Center: Lima, Peru

Hedr´en, E., Diaz, V., & Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. European Journal of Clinical Nutrition, 56(5):425–30

Kean, E.G., Hamaker, B.R., & Ferruzzi, M.G. (2008). Carotenoid bioaccessibility from whole grain and degermed maize meal products. Journal of Agricultural and Food Chemistry, 56, 9918–9926

Kidmose, U., Yandg, R.Y., Thilsted, S.H.; Christensen, L.P., & Brandt, K. (2006) Content of carotenoids in commonly consumed Asian vegetables and stability and extractability during frying. Journal of Food Composition and Analysis, 19, 562–571

Lipkie, T.E., De Moura, F.F., Zhao, Z.Y., Albertsen, M.C., Che, P., Glassman, K., & Ferruzzi, M.G. (2013). Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. Journal of Agricultural Food Chemistry, 61, 5764−5771

Nagao, A., Nara, E. & Hase, M. (2013). Effects of fats and oils on the bioaccessibility of carotenoids and vitamin E in vegetables. Journal of Bioscience, Biotechnology, and Biochemistry, 77:5, 1055-1060, DOI: 10.1271/bbb.130025. https://doi.org/10.1271/bbb.130025

Pugliese, A. O'Callaghan, Y., Tundis, R., Galvin, K., Menichini, F., O'Brien, N. & Loizzo, M. R. (2013). The effect of domestic processing on the content and bioaccessibility of carotenoids from chili peppers (*Capsicum* species). Journal of Plant Foods and Human. Nutrition, 69, 8–17

Thakkar, S.K., & Failla, M.L. (2008). Bioaccessibility of pro-vitamin A carotenoids is minimally affected by non pro-vitamin A xanthophylls in maize (*Zea mays sp*.). Journal of Agricultural and Food Chemistry, 56, 11441–11446

Veda, S., Patel, K., & Srinivasan, K. (2010). Enhanced bioaccessibility of beta-carotene from yellow-orange vegetables and green leafy vegetables by domestic heat processing. International Journal of Food Science and Technology, 45, 2201–2

Table 4-1. Contents of all-trans, 13-cis and 9-cis β-carotenes (μg/g on a Fresh Weight Basis) in the OFSP puree/flour and maize /wheat flour blends before cooking

Sample	13-cis	All-trans	9-cis	Total	Moisture content
50% vita puree+50% maize flour	5.26±0.57^{a}	21.62±2.18^{a}	0.51±0.12^{a}	26.20±3.20^{a}	60.8±1.09^{b}
50% kabode puree+50% maize flour	3.37±0.50^{a}	17.31±1.67^{a}	0.42±0.10^{a}	22.39±2.70^{a}	60.6±0.66^{b}
50% vita flour+50% maize flour	4.85±0.06^{a}	25.30±0.34^{a}	0.57±0.08^{a}	30.89±1.50^{a}	6.5±0.07^{a}
50% kabode flour+50% maize flour	4.06±0.20^{a}	23.56±0.63^{a}	0.76±0.11^{a}	27.50±1.65^{a}	6.5+0.17^{a}
50% vita puree+50% wheat flour	5.64±0.30^{ab}	21.17±0.67^{a}	0.59±0.01^{a}	28.14±1.27^{a}	60.6±0.61^{b}
50% kabode puree+50% wheat flour	4.04±0.20^{a}	14.37±0.30^{a}	0.45±0.23^{a}	18.33±1.24^{a}	59.6±0.43^{b}
50% vita flour+50% wheat flour	8.38±1.14^{bc}	44.50±1.20^{b}	1.40±0.20^{b}	55.62±2.12^{b}	6.6±0.17^{a}
50% kabode flour+50% wheat flour	8.42±0.70^{c}	43.15±5.50^{b}	1.50±0.11^{b}	51.55±4.87^{b}	6.3±0.04^{a}

Data represent mean ± SEM; $n = 5$. Letters with different superscripts represent significant differences ($P < 0.05$) between samples within individual species and total carotenoid using Tukey's test.

Table 4.2. Total B-carotene, all-trans β-carotene, 13-cis β-carotene and 9-cis β-carotene in porridge and *chapati* containing OFSP puree and flour (µg/g FW)

Genotype	Product	13-cis	All-trans	9-cis	Total	Moisture content
Vita	Puree porridge	3.74±0.40^{a}	16.69±1.78^{ab}	0.359±0.08^{a}	20.79±0.22^{ab}	85.8±0.23^{c}
	Flour porridge	3.36±0.12^{a}	19.63±0.33^{b}	0.459±0.05^{a}	23.44±0.24^{b}	85.7±0.01^{c}
	Puree chapati	4.40±0.06^{ab}	17.46±0.26^{ab}	0.441±0.00^{a}	22.29±1.01^{ab}	33.0±0.22^{b}
	Flour chapati	6.51±0.82^{c}	37.91±1.81^{c}	1.003±0.14^{b}	45.42±0.09^{c}	24.7±0.14^{a}
Kabode	Puree porridge	2.51±0.42^{a}	13.33±1.27^{ab}	0.324±0.07^{a}	16.16±1.67^{ab}	85.2±0.01^{c}
	Flour porridge	3.01±0.14^{a}	17.61±0.78^{ab}	0.561±0.07^{a}	21.18±0.95^{ab}	86.0±0.22^{c}
	Puree chapati	3.06±0.23^{a}	11.64±0.47^{a}	0.345±0.02^{a}	15.05±0.48^{a}	28.2±0.22^{a}
	Flour chapati	6.39±0.50^{bc}	36.22±3.2^{c}	1.175±0.10^{b}	43.78±3^{c}	29.3±0.01^{ab}
Control	Maize flour porridge	ND	ND	ND	ND	-
Control	Wheat flour porridge	ND	ND	ND	ND	-

Abbreviation: ND = Not detected. Data represent mean ± SEM; n = 5. Letters with different superscripts represent significant differences ($P < 0.05$) between samples within individual species and total carotenoid using Tukey's test.

Table 4-3. Total B-carotene, all-trans β-carotene, 13-cis β-carotene and 9-cis β-carotene in porridge and *chapati* containing OFSP puree and flour (μg/g DW)

Genotype	Product	13-cis	All-trans	9-cis	Total
Vita	Puree porridge	3.74 ± 0.32^{a}	116.1 ± 12.34^{cd}	2.49 ± 0.56^{bcd}	144.6 ± 15.48^{d}
	Flour porridge	3.36 ± 0.13^{a}	135.8 ± 2.27^{d}	3.18 ± 0.37^{cd}	162.2 ± 1.64^{d}
	Puree chapati	6.54 ± 0.10^{bc}	26.0 ± 0.40^{ab}	0.66 ± 0.01^{a}	33.2 ± 0.45^{ab}
	Flour chapati	8.64 ± 1.10^{c}	50.4 ± 2.40^{b}	1.33 ± 0.18^{ab}	60.3 ± 1.34^{b}
Kabode	Puree porridge	2.51 ± 0.42^{a}	89.1 ± 8.46^{c}	3.93 ± 0.44^{d}	108.0 ± 11.15^{c}
	Flour porridge	3.01 ± 0.14^{a}	123.3 ± 5.43^{d}	2.16 ± 0.51^{abc}	148.3 ± 6.68^{d}
	Puree chapati	4.28 ± 0.32^{ab}	16.3 ± 0.65^{a}	0.48 ± 0.03^{a}	21.1 ± 0.67^{a}
	Flour chapati	9.04 ± 0.71^{c}	51.3 ± 4.55^{b}	1.66 ± 0.13^{abc}	62.0 ± 5.38^{b}
Control	Maize flour porridge	ND	ND	ND	ND
Control	Wheat flour porridge	ND	ND	ND	ND

Abbreviation: ND = Not detected. Data represent mean ± SEM; $n = 5$. Letters with different superscripts represent significant differences ($P < 0.05$) between samples within individual species and total carotenoid using Tukey's test

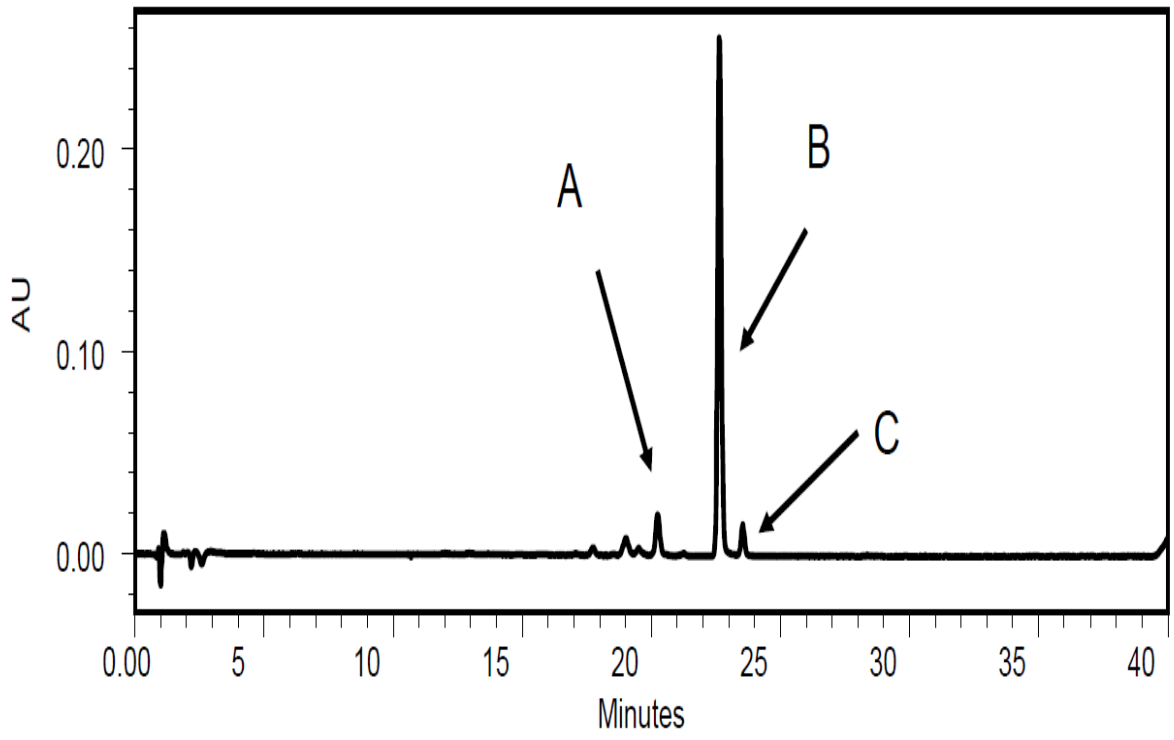

Figure4-1. Chromatograms (abs 450 nm) of major provitamin A carotenoids found in OFSP-maize flour porridge and OFSP-wheat flour *chapati*. Peak identification: 13-cis β-carotene (A), all-trans β-carotene (B), 9-cis β-carotene (C).

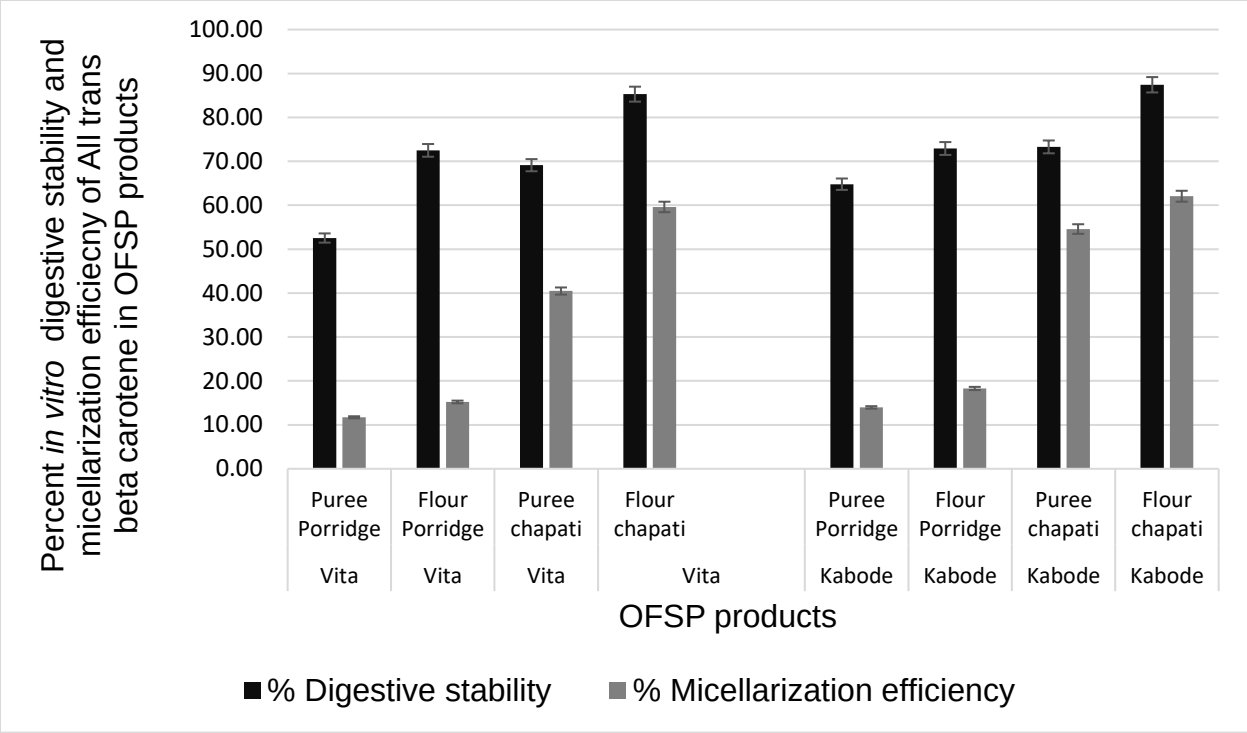

Figure 4-2. Effect of processing methods on the in vitro β-carotene bioaccessibility of OFSP products. Values are presented as mean ± SEM; n = 3.

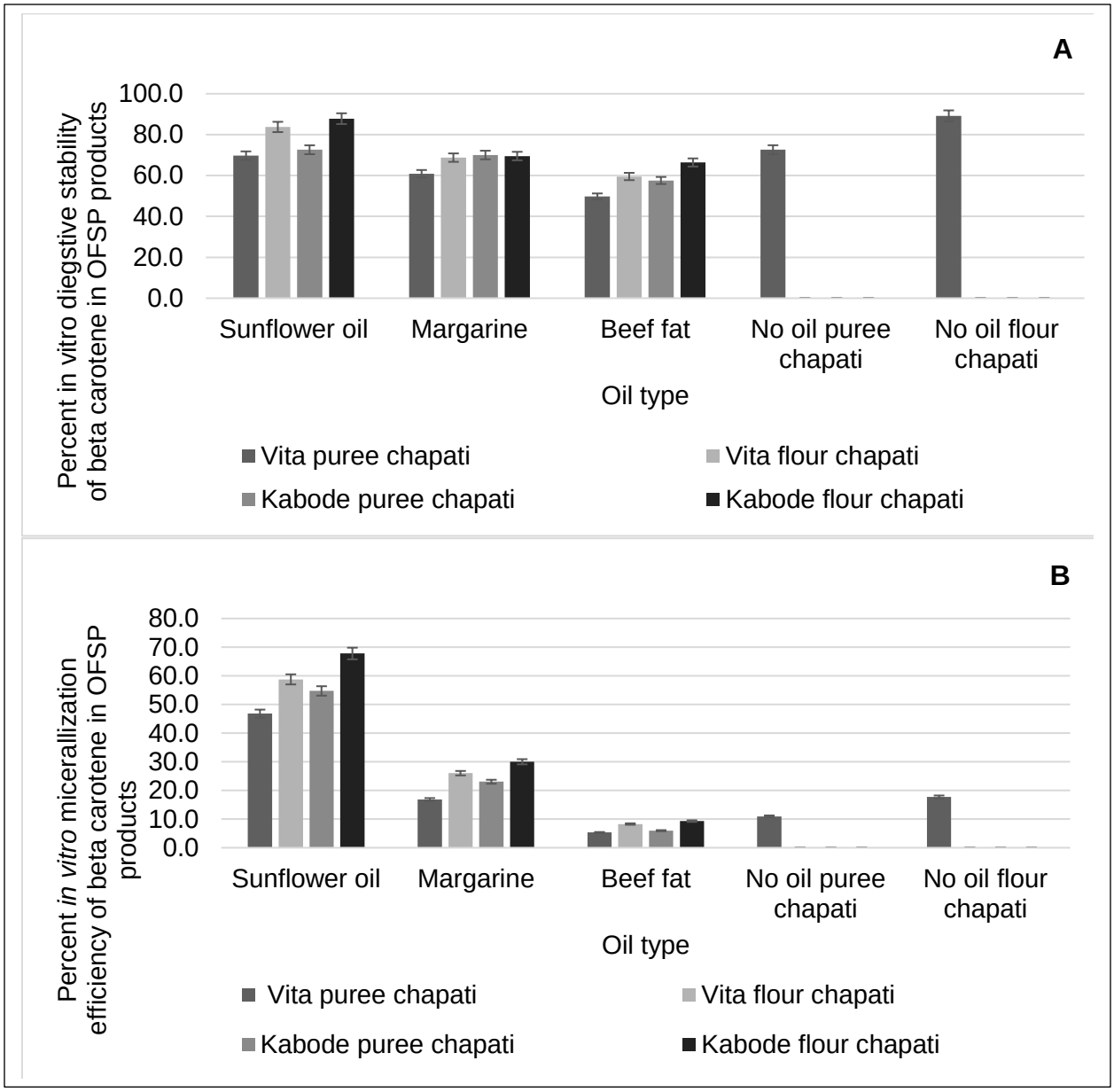

Figure 4-3. Effect of oil type on bioaccessibility of all-trans β-carotene in OFSP products. Digestive stability (A), Micellarization efficiency (B). Values are presented as mean ± SEM; n = 3.

CHAPTER 5.

BIOCONVERSION EFFICIENCY OF β-CAROTENE FROM ORANGE-FLESHED SWEETPOTATO PRODUCTS INTO VITAMIN A

Bioconversion Efficiency of β-Carotene from Orange-Fleshed Sweetpotato products into Vitamin A

Abstract

Orange-fleshed sweetpotatoes (OFSP) contain significant quantities of provitamin A carotenoids. Determination of conversion efficiency of β-carotene from OFSP into vitamin A (VA) is important to determine its effectiveness as source of VA in humans. The objectives of the study were to identify products of β-carotene cleavage and determine the conversion rate of β-carotene into retinoid. Beta-carotene extracts from OFSP supplemented products were incubated with chicken intestinal mucosa post mitochondrial fractions for 60 min at 37°C. Pure all-trans β-carotene standard with and without chicken intestinal mucosa post mitochondrial fractions were incubated as controls. HPLC analysis was performed to identify β-carotene cleavage products. When β-carotene extract was incubated with chicken intestinal mucosa post mitochondrial fractions retinal (RAL) and retinoic acid (RA) were formed. No RAL and RA were formed when pure all-trans β-carotene standard was incubated without chicken intestinal mucosa post mitochondrial fraction. On average, the conversion ratio of β-carotene to RAL was 5:1. The results are of significance to people in developing countries who depend on plant food to meet their VA requirements.

Keywords: BCMO1, Cleavage, β-carotene, Vitamin A, OFSP

5.0. Introduction

All-trans β-carotene is a pro-vitamin A molecule that is converted into two molecules of VA by the enzyme called 15, 15'-dioxygenase (BCMO1). In humans, the bioconversion of β-carotene into VA takes place mostly in the small intestines under central cleavage pathway (Goodman et al., 1996).The enzyme 15, 15'-dioxygenase cleaves β-carotene at central double bond into two molecules of retinal. Retinal acts as an intermediate product of β-carotene cleavage as it is exclusively metabolized into retinoic acid (Yuem et al., 2000; Napoli & Race, 1988). Conflicting reports on the fate of retinal indicate that it is converted into retinol and retinoic acid through reduction and oxidation processes respectively (Oslon & Hayaishi, 1965). There exists evidence of eccentric cleavage of β-carotene through which β-apo-carotenoids lacking VA activity are produced (Russell, 2004; Krinsky et al., 1993). It is widely known that the enzyme 15, 15'-dioxygenase cleaves only all-trans β-carotene. The remaining carotenoids are incorporated intact in chylomicrons together with lipids and circulate in association with VLDL, LDL, and HDL and hence can be taken up by the respective receptors for these lipoproteins (Castenmiller & West, 1998). The major factors that affect the bioavailability of food carotenoids and the bioconversion of food provitamin A carotenoids to vitamin A in humans are food matrices, food preparation, and the fat content of a meal (Furr and Clark, 1997; West, and Castenmiller, 1998).

In the body VA is mainly stored in the liver prior to distribution to various organs and tissues where it is particularly essential for normal growth and development, immune function, and vision (WHO, 2009). Preformed VA is present in animal products only while pro-vitamin A is found in carotene rich plants. Vitamin A deficiency is a condition resulting from inadequate intake of VA. Preformed VA is more bioavailable compared to pro-vitamin A, thus, putting

people who depend on plant foods as the only source of VA at risk of VAD (Greiner, 2013). OFSP is a biofortified crop rich in pro-vitamin A hence a safe and sustainable tool against VAD. Over the years, OFSP based products have been developed in Africa and are widely consumed as a sustainable strategy to tackle VAD. Although consumption of carotene rich foods like OFSP is being promoted, little is known about the bioconversion efficiency of β-carotene into retinol of such foods. It is therefore important to determine the conversion efficiency of β-carotene into VA through *in vitro* methods. As a continuation of β-carotene *in vitro* bioaccessibility part of this thesis, an *in vitro* bioconversion experiment was performed to identify cleavage products of β-carotene and determine the conversion rate of β-carotene into retinoid. The methods described by During et al. (1996) and Yuem et al. (2000) was adopted with minor modifications for the experiment. The protocol was comprised of three parts including extraction of the enzyme from chicken intestines, incubating the carotene extract with enzyme for β-carotene cleavage and retinoid analysis by HPLC as described below;

5.1. Materials and methods

5.1.1. Enzyme preparation

The upper half of chicken intestines was washed with ice-cold isotonic saline solution (0.9% NaCl). The intestinal mucosa was gently scraped off with a razor blade and homogenized with 50 mM HEPES buffer, pH 7.4 containing 1.15% KCl, 1 mM EDTA and 0.1 mM 2-mercapto ethanol. The post nuclear fraction was prepared by centrifuging the intestinal homogenate at 800 x g for 30 min. The fraction was centrifuged further at 10,000 x g for 1 h at 4°C to remove particulate matter and some organelles. The protein concentration of the protein

fraction (enzyme) was determined by the Bradford method. The protein fractions were loaded in amber vials, purged with nitrogen and kept at -80°C.

5.1.2. Incubation of β-carotene with enzyme

The pre-incubation mixture contained 13 mg protein fraction and 300 µL buffer solution (0.1 mM α-tocopherol, 0.1 M tricine buffer, pH 8.0, 6 mM sodium taurocholate and 0.5 mM 2-mercapto ethanol). Alpha-tocopherol was added to prevent oxidation of β-carotene and promote its conversion into VA as described by Yuem et al. (2000). The volume of protein fraction was slightly increased as opposed to previous authors (During et al., 1996 and Yuem et al., 2000) due to low protein concentration of the enzyme. The mixture was incubated at 37^{o}C in a shaking water bath for 5 min. After incubation, the enzyme reaction was initiated by adding 100 µg of β-carotene solubilized in aqueous 167 µL of 4.5% Tween 40 in acetone and incubated at 37^{o}C for 1 h. The enzyme reaction was terminated by adding 100 µL of 37% formalin solution (w/w) and incubated at 37^{o}C for 10 min. Samples of pure all-trans β-carotene with and without protein fractions, and protein fractions only were run as controls.

5.1.3. Retinoid extraction of cleaved β-carotene

Retinoid extraction was according to During et al. (1996) and Yuem et al. (2000) with some modifications. The incubation mixture was extracted with 1.5 mL of chloroform:methanol (2:1, v/v) + 0.5 mL hexane. The mixture was placed in ice for 5 min and then insoluble matter was removed by centrifuging at 10,000 g, 4^{o}C for 10 min. The step was repeated twice to ensure complete extraction of retinoid. The β-carotene extract was evaporated to complete dryness under nitrogen and solubilized with 150 µL of ethanol and run on HPLC to determine amount of

RAL, and RA formed. The experiment was carried out in yellow light to minimize carotenoid isomerization and degradation by light irradiation.

5.1.4. HPLC analysis of retinoid

The formation of retinoid was monitored by HPLC equipped with a pump, a degaser and a UV6000LP photodiode array detector. Retinoids were eluted on a TSK gel ODS-80Ts C18 reverse phase column, 4.6 X 150 mm connected to pre-column (2 x 20 mm) of Pelliguard LC-18 (Sigma-Aldrich, 13570 Stockholm, Switzerland). Mobile phase A contained acetonitrile: THF: H20 (50:20:30, v/v/v, 1% ammonium acetate) while mobile phase B contained acetonitrile: THF: H20 (50:44:6, v/v/v, 1% ammonium acetate). The gradient procedure at a flow rate of 0.4 mL/min was as follows: 100% solvent A for 3 min followed by a 9 min linear gradient to 40% A and 60% B, with a 12 min hold, then a 6 min gradient back to 100% solvent A.

5.2. Results and discussions

5.2.1. Identification of products of β-carotene cleavage

The HPLC chromatogram of β-carotene cleavage products from OFSP products is shown in Figures 5-1 and 5-2. Retention times and absorption spectra of authentic standards were used to identify the retinoids. The absorption spectra for RAL and RA was 380 nm and 340 nm respectively. According to the method developed in this study, the retention times for RA was 7 min and 14 min for RAL. Quantification of carotenoids was achieved using a calibration curve with the correlation coefficient of $\geq$0.998. According to Figures 5-1 and 5-2, RAL and RA were the main retinoids identified as β-carotene cleavage products from β-carotene extracts incubated

with intestinal homogenate. No β-carotene cleavage products were detected from β-carotene extracts incubated without intestinal homogenate.

5.2.2. Bioconversion efficiency of β-carotene into retinoid

In vitro bioconversion of β-carotene into retinoid was performed using chicken intestinal homogenate. When OFSP products were incubated with post mitochondrial fraction of chicken intestinal mucosa homogenate in the presence of α-tocopherol, central cleavage products, retinoic acid (RA) and retinal (RAL) were observed but not retinol (ROH). There were significant differences in the amount of RA and RAL formed between the products, although similar products had comparable amounts of RA and RAL. The amount of RAL formed ranged from 12.06 to 29.92 μg/mg protein while RA ranged from 23.34 to 40.34 μg/mg protein. As expected pure all-trans β-carotene had the highest content of RAL (40.14 μg/mg protein) and RA (62.01 μg/mg protein). When pure all-trans β-carotene was incubated without intestinal homogenate, no central cleavage products were observed. The finding confirms the efficacy of chicken intestinal mucosa post mitochondrial fraction homogenate in converting β-carotene into VA active compounds. The difference in RAL and RA formed between porridge and *chapati*, confirms the influence of food matrix on conversion of β-carotene into RAL or RA as reported by West and Castenmiller (1998). Addition of oil in *chapati* might have also increased β-carotene conversion efficiency into VA compared to porridge. The bioconversion efficiency of β-carotene was higher when rats were fed with oily solution of β-carotene (Tanumihardjo, 2002). From the results, the amount of RA formed was higher than RAL indicating that most of RAL was converted into RA. The conversion of RAL into RA suggests presence of aldehyde dehydrogenases (NAD^+) and alcohol dehydrogenases enzymes in the post mitochondrial

fraction. Napoli and Race (1988) did not detect RAL as an intermediate of β-carotene conversion into RA probably due to high concentration of NAD^+. With reference to the β-carotene concentration of the products (100 μg), on average the conversion ratio of β-carotene to RAL is 5:1. The conversion ratio of β-carotene into RAL found by this study are slightly higher than 12:1 estimated by Food and Nutrition Board (IOM, 2001). Previous studies also reported high bioconversion rates of β-carotene to retinol, with reported values of 7:1 (Li et al., 2010) and 3:1 (Muzhingi et al., 2011) for biofortified maize. The difference between the conversion ratios might be due to differences in methods used to determine β-carotene conversion efficiency into VA. The current study used an *in vitro* method to determine the conversion ratio while Food and Nutrition Board (IOM, 2002) used an *in vivo* method, hence the difference.

Previous studies that used pure BCMO1 enzyme or purified post mitochondrial factions of intestinal homogenate for β-carotene incubation, reported retinal as the only product of β-carotene cleavage (Oslon and Hayaishi, 1965; Nagoa et al., 1996). In contrast, Yuem et al. (2000) reported RAL and RA as main products of β-carotene cleavage using rat intestinal homogenate. In the current study, no eccentric oxidative cleavage products such as apocarotenal and apocarotenone were observed. This can be explained by inhibition effect of α-tocopherol on formation of eccentric cleavage oxidative products. The finding agrees with findings by Yeum et al. (2000) who reported that presence of α-tocopherol prevents conversion of β-carotene into eccentric cleavage oxidative products like apocarotenal and apocarotenone. Liu et al. (2004) reported that combination of α-tocopherol and ascorbic acid had a stronger effect on the inhibition of the smoke-enhanced formation of eccentric cleavage oxidative metabolites of α-carotene, and on the restoration of the smoke-reduced production of central cleavage metabolites of β-carotene than did either α-tocopherol or ascorbic acid alone. Formation of eccentric

cleavage oxidative products facilitates formation of cancer cells. On the other hand, RA plays an important role in modulating cell proliferation and differentiation of lung epithelial cells, and suppresses carcinogenesis in certain epithelial tissues (Lippman & Lotan, 2000).

5.3. Conclusion

The study found that β-carotene extracts of OFSP products were successfully converted into RAL and RA after incubating with chicken intestinal homogenate. The conversion rate of β-carotene into RAL found by this study is relatively high compared to literature. Still, the findings are of significance to people in developing countries who depend on plant food to meet their VA requirements. This was a one-time experiment and results might not be applicable to the whole organism. In addition, the limitation of the study lies on use of *in vitro* methods to determine bioconversion rate of β-carotene into RAL. Thus, *in vivo* studies are suggested to validate the findings.

REFERENCES

Castenmiller , J. J, & West C. E. (1998). Bioavailability and bioconversion of carotenoids. Journal of Annual Review in Nutrition. 1998;18:19–38

During A., Nagao, A, Hoshino C., and Terao J. (1996). Assay of β-Carotene 15, 15'-dioxygenase activity by reverse-phase high-pressure liquid chromatography. Journal of Analytical Biochemistry, 241: 199–205

During, A., Albaugh, G. and Smith, Jr. J.C. (1998). Characterization of β-carotene 15, 15'-dioxygenase activity in TC7 clone of human intestinal cell Line Caco-2. Journal of Biochemical and Biophysical Research Communications, 249: 467–474

Food and Nutrition Board, Institute of Medicine. (2001) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A report of the Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Washington, DC: National Academy Press, 2001

Furr, H. C & Clark, R. M. (1997). Intestinal absorption and tissue distribution of carotenoids. Journal of Nutrition Biochemistry, 8:364–77

Greiner, T. (2013). Vitamin A: Moving the food-based approach forward. ICN2 second international conference on nutrition: better nutrition better lives. http://www.fao.org/publications. Accessed on December 21, 2018

Li, S., Nugroho, A., Rocheford, T. & White, W. S. (2010). Vitamin A equivalence of the β-carotene in β-carotene-biofortified maize porridge consumed by women. American Journal of Clinical Nutrition, 92(5):1105–1112

Lippman, S. M. & Lotan, R. (2000). Advances in the development of retinoids as chemopreventive agents. Journal of Nutrition, 130: 479S–482S

Liu, C., Robert, M. Russell, & Xiang-Dong Wang. (2004). α-Tocopherol and ascorbic acid decrease the production of β-apo-carotenals and increase the formation of retinoids from β-carotene in the lung tissues of cigarette smoke–exposed ferrets *in vitro*. Journal of Nutrition, 134: 426–430

Muzhingi, T., Gadaga, T. H., Siwela, A. H., Grusak, M. A., Russell, R. M. & Tang, G. (2011). Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men. American Journal of Clinical Nutrition, 94(2):510–519

Napoli, J. L. & Race, K. R. (1988). Biogenesis of retinoic acid from β-carotene. Journal of Biology and Chemistry, 263**:**17372–17377

Oslon, J. A. & Hayaishi, O. (1965). Proceedings of the National Academy of Science in United States of America, 54:1364-1370

Tanumihardjo, S. A. (2002). Factors influencing the conversion of carotenoids to retinol: Bioavailability to bioconversion to bioefficacy. International Journal of Vitamin and Nutritional Research, 72: 40-45

Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M. & Grusak, M. G. (2005). Spinach or carrot can supply significant amounts of vitamin A as assessed by feeding with intrinsically Deuterium-labeled vegetables. American Journal of Clinical Nutrition, 82: 821-8

Wang, X. D., Tang, G., Fox, J. Krinsky, N. I. & Rusell, M. (1991). Enzymatic conversion of β-carotene into β-apo-carotenals and retinoids by human, monkey, ferret, and rat tissues. Archives of Biochemistry and Biophysics, 258: 8-16

West, C. E. & Castenmiller, J. J. M. (1998). Quantification of the "SLAMENGHI" factor for carotenoid bioavailability and bioconversion. International Journal of Vitamin and Nutrition Research, 68: 371-377

World Health Organization and Food and Agriculture Organization of the United Nations. (2004). Vitamin and mineral requirements in human nutrition. Report of joint FAO/WHO expert consultation Bangkok, Thailand

World Health Organization. (2009). Global prevalence of vitamin A deficiency in populations at risk 1995–2005. In: WHO Global Database on Vitamin A Deficiency. WHO, Geneva, Switzerland

Yeum K. J. Ferreira dos A. A. L., Smith D, Krinsky N. I. & Russell R. M. (2000).The effect of α-Tocopherol on the oxidative cleavage of β-Carotene. Journal of Free Radical Biology and Medicine, 29(2):105-14

Table 5-1. The production of retinoids after incubation of 100 µg β-carotene extract with mitochondrial fraction of chicken intestines

Sample	RAL	RA
	(µg/mg protein/60 min incubation)	
Vita puree porridge + homogenate	12.87±0.6a	23.34±1.1a
Kabode puree porridge + homogenate	12.06±0.5a	23.82±0.4ab
Vita flour porridge + homogenate	15.32+0.2ab	25.95±0.5ab
Kabode flour porridge + homogenate	15.23±0.2ab	25.56±0.6ab
Vita puree chapati + homogenate	18.65±0.6b	29.69+1.4ab
Kabode puree chapati + homogenate	17.38±1.1b	30.19±1.8b
Vita flour chapati + homogenate	28.62±1.5c	39.07±1.8c
Kabode flour chapati + homogenate	29.92±5.2c	40.34±1.4c
β-carotene standard + homogenate	40.14±0.7d	62.01±1.89d
β-carotene standard - homogenate	ND	ND
Homogenate	ND	ND

Data represent mean ± SEM; $n = 5$. Letters with different superscripts represent significant differences ($P < 0.05$) between samples within individual species and total carotenoid using Tukey's test. ND = not detected

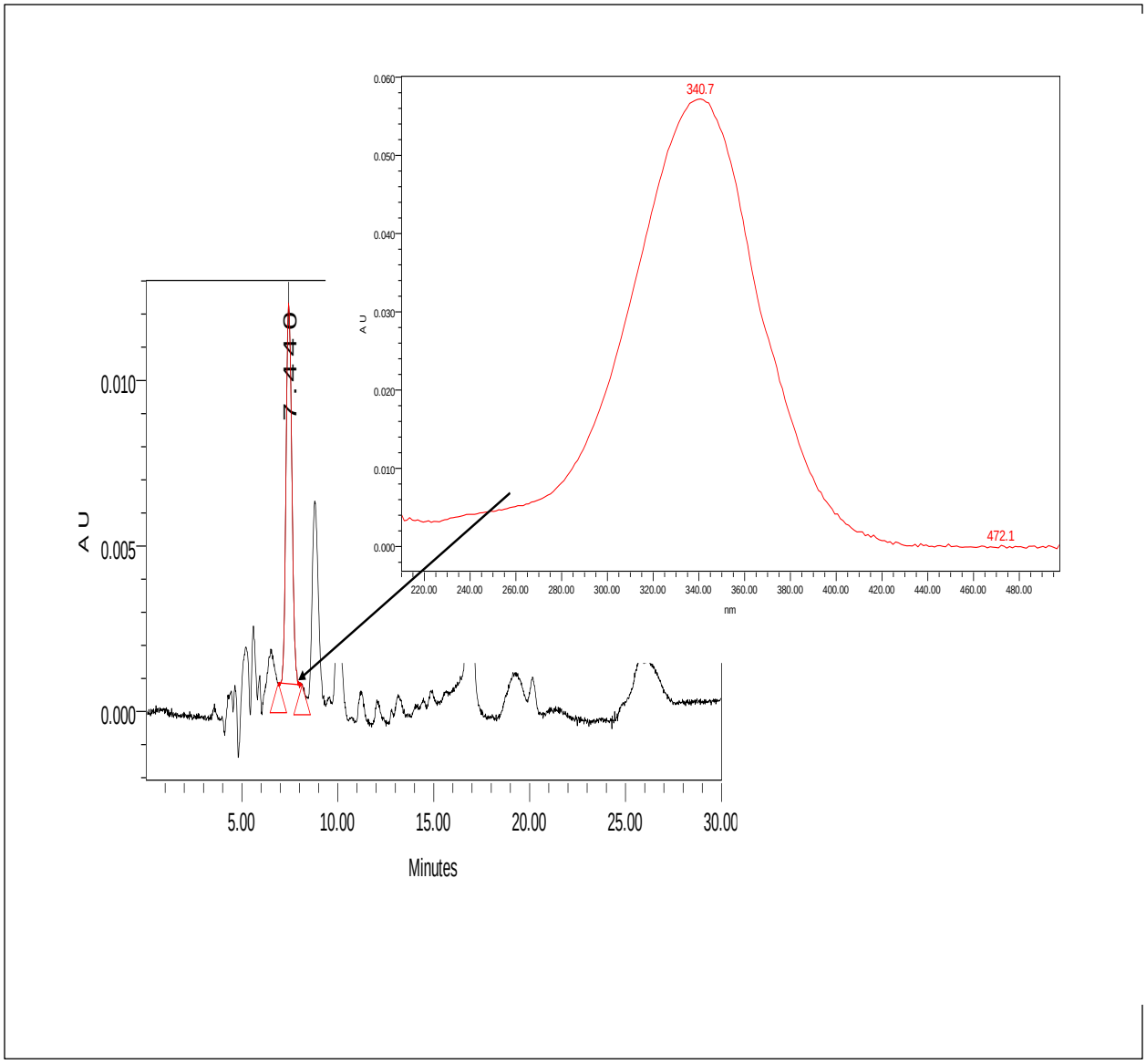

Figure 5-1. Retinoic acid peak (at 340 nm) of chapati β-carotene extract with chicken intestinal homogenate

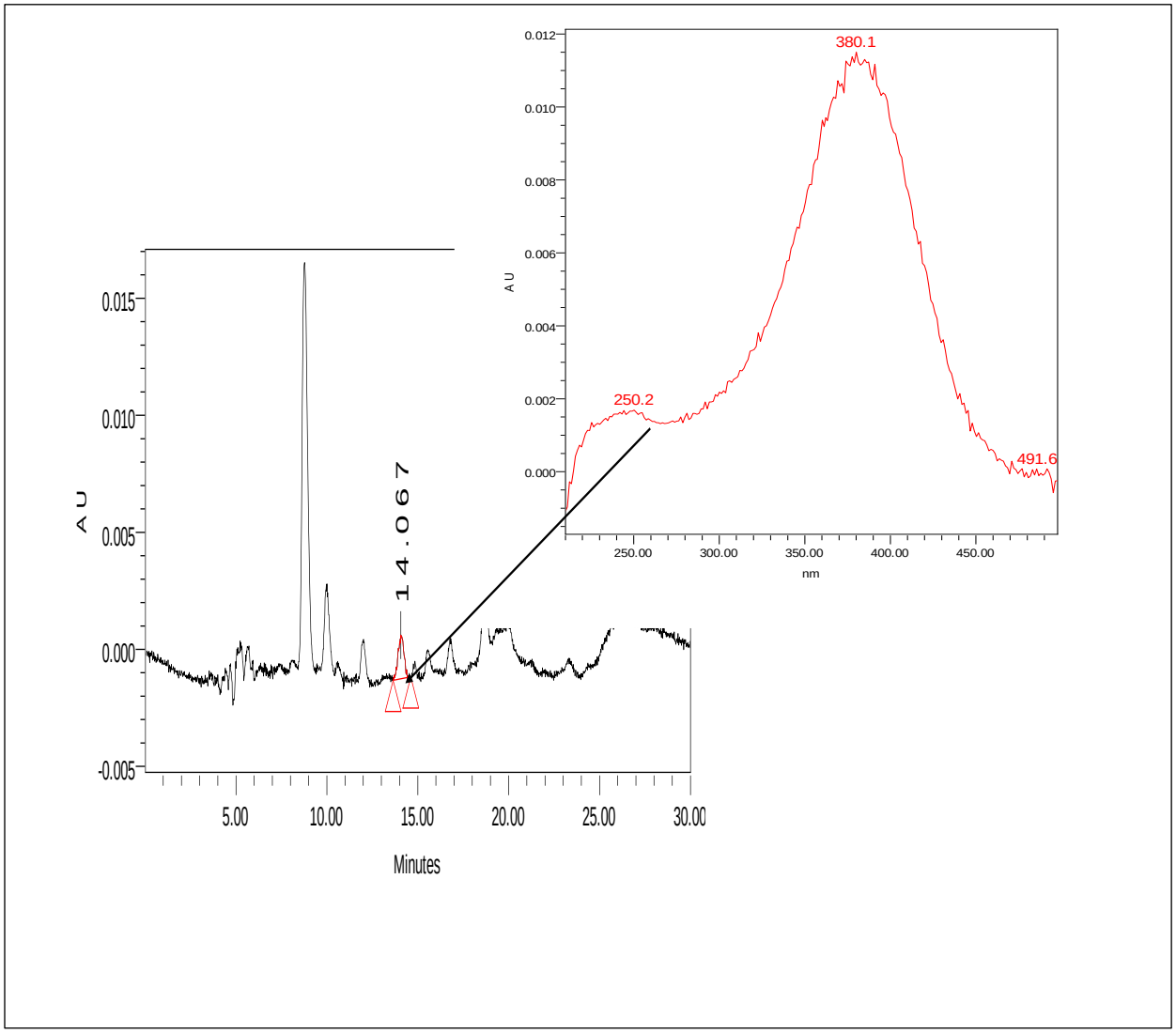

Figure 5-2. Retinal peak (at 380 nm) of *chapati* β-carotene extract with chicken intestinal homogenate

CHAPTER 6.

CONCLUSIONS AND SUGGESTED FUTURE RESEARCH WORKS

6.0. Summary

In the recent past OFSP is being used as a strategy to tackle VAD in Africa. Scarcity of information on carotene bioaccessibility of traditionally processed OFSP products is a limiting factor in promotion of the crop to tackle VAD. The main objective of the thesis was to evaluate the effect of traditional processing on carotene bioaccessibility. It was hypothesized that OFSP can be processed into products with high carotene content and bioaccessibility. The main achievement of the research study was quantification of bioaccessible β-carotene from OFSP supplemented products. Micellarization efficiency of β-carotene in OFSP products was calculated as a measure of relative bioaccessiblity. It was found that *chapatis* had high β-carotene bioaccessibility compared to porridge due to addition of oil in the formulation. The products (porridge and *chapati*) were supplemented with either OFSP puree or flour. From the results flour-based products had significantly higher bioaccessible carotene than puree based products. The results were related to maceration effect of flour processing that impacted cell disruption making β-carotene more accessible to digestive enzymes. Roasting of *chapatis* at high temperature (175^{o}C) also improved carotene bioaccessibility.

Furthermore, the study evaluated the effect of oil type on β-carotene bioaccessibility. Three oils types namely sunflower oil, margarine and beef fat were evaluated on carotene bioaccessibility of OFSP *chapatis*. It was found by the study that sunflower oil had optimal carotene bioaccessibility followed by margarine while beef fat was the least. The results were explained by the low melting point of sunflower oil, thus solubilizing carotene at the digestion temperature. Margarine and beef fat have high proportions of saturated fats characterized by high melting points hence not effective at solubilizing carotene during digestion to facilitate its incorporation into the mixed micelles. Pure sunflower oil is readily available in Africa and

consumers could take advantage of that to add in *chapati* formulation and increase β-carotene bioaccessibility. Thus, it is concluded that processing methods with substantial degree of cell disruption and presence of polyunsaturated oil in the formulation are critical to enhance carotene bioaccessibility from OFSP products.

Bioconversion efficiency of β-carotene into VA was determine in this study through *in vitro* method using BCMO1 obtained from chicken intestines. According to HPLC analysis of retinoids, RA and RAL were identified as main products of β-carotene conversion into VA. Although the OFSP products had different conversion rates, on average the conversion ratio of β-carotene to RAL was 5:1. The bioconversion efficiency found by this study is high compared to 12:1 reported by Institute of Medicine (OIM, 2001). Replication of the study is suggested to validate the findings.

OFSP flour is still the primary product used in product formulation. Several studies have reported high carotenoid loss of dried stored carotene rich products. The second achievement of the thesis is identification of packaging material of OFSP flours with highest carotene retention during 4 months storage period. It was found that AFL coupled with vacuum sealing was effective at retaining carotene of stored OFSP flours and results were neither dependent of storage environment (light and dark) nor genotype. The results confirmed that oxidation and not light was the main factor for carotene degradation of stored OFSP flour. Apart from light, color and water activity were also assessed as fundamental factors influencing carotene content of stored OFSP flours. There was a slight change in color parameters (a*, b* and L) of stored OFSP flours overtime. Correlation of a* and β-carotene content showed weak association between the two. A slight but significant increase in water activity of flours was observed during 4 months storage period. Furthermore, water activity strongly and positively correlated with carotene

content of the stored flours. In brief, color, particularly a* and water activity can be used as a measure of carotene content of stored OFSP flour. In conclusion, the carotene loss of stored OFSP flours was determined. Degradation of carotene in stored OFSP flours is the major setback as low carotene content of flour translates to inadequate carotene being delivered after consumption. Therefore, OFSP flour processors need to consider the packaging material for maximum carotene retention.

6.1. Suggested future study

Future research work is needed particularly on minimizing carotenoid losses during processing and exploring ways to improve carotene bioaccessibility of OFSP products. Comprehensive studies have been done on modeling carotenoid loss during drying and storage of dried OFSP products. The same can be done on modeling carotene losses during thermal processing of OFSP based products for improved carotene bioaccessibility. Research questions to address various research gaps are below;

- What is the relationship between sweetpotato genetic makeup and β-carotene bioaccessibility? Although this thesis did not establish the effect of genotype on carotene retention and bioaccessibility probably due to few genotypes evaluated, it is important to undertake a comprehensive study on the same. Wide range of OFSP genotypes have been developed and released to the community for adoption in the recent past. It is worthwhile to study the relationship between genetic makeup and carotene bioaccessibility of OFSP. The study will generate information important to plant breeders to improve breeding

programs to breed OFSP genotypes with high carotene bioaccessibility and help in the fight of VAD.

- What is the carotenoid degradation pathway of thermally processed OFSP products? Bechoff et al. (2010), developed and reported the kinetics (*k*) of carotenoid degradation of dried OFSP chips but not heat processed products like porridge and *chapati*. The authors reported that β-carotene degradation followed first order reactions of kinetic modeling. In a separate study, Achir et al. (2013) observed that temperature had a major influence on reaction rates *k* while water activities only impacted *k* at values under 0.51. The use of multi-response modelling was used by Achir et al. (2011) to improve the understanding of degradation of β-carotene in oil. However, the studies did not account for the factors responsible for carotene degradation during thermal processing to develop mathematical models. Kinetic models involving temperature, moisture content and oxygen would help in developing mathematical models to determine carotene loss and formation of products during thermal processing. Special focus on products of carotenoid degradation during thermal processing is also important. Moreover, the products generated during β-carotene degradation may have positive or negative biological activity (Zhang and Omaye 2001). Comprehension of the processes will assist in developing novel ways to minimize carotenoid degradation during thermal processing.

- What is the β-carotene bioavailability of OFSP products commonly consumed in Africa to help eradicate VAD? Bioavailability is defined as the fraction of carotenoid that is absorbed and available for utilization in normal physiological functions or for storage

(Tanumihardjo, 2002). The current study did not undertake bioavailability of β-carotene in OFSP products. It is categorically important to determine bioavailability of β-carotene to determine the fraction that it is absorbed and made available for body utilization. Thus, bioavailability of β-carotene using Caco-2 cells is suggested.

- What are the various micronutrients affecting β-carotene bioavailability and bioconversion of OFSP products? How does the interaction between the micronutrients affect carotene bioavailability and bioconversion? The factors affecting carotenoid bioavailability have been discussed extensively in the literature and can be summarized in the mnemonic SLAMENGHI proposed by West and Castenmiller (1998). Apart from carotenes, OFSP contain significant quantities of vitamin C, iron, zinc, anthocyanins and antinutrient like phytates. Previous study indicated micronutrient bioavailability is impacted by antinutrients. Interaction between micronutrients and their effect on nutrient bioavalability has been reported by Bechoff and Dhuique-Mayer (2017). Yuem et al. (2000) reported that the presence of α-tocopherol improved carotene bioconversion. Understanding the effect of various micronutrients and their interactions on carotene bioavailability and bioconversion is essential to determining processing methods that will guarantee maximum carotene bioavailability and bioconversion.

The study has evaluated the effect of processing on bioaccessibility of β-carotene. Vitamin A deficiency is still a major micronutrient deficiency problem in sub-Saharan Africa. The information generated by this research work will contribute in fight against VAD as it helps in understanding processing methods with optimal β-carotene bioaccessibility. The information will also help plant breeders to continue breeding for OFSP through biofortification. While

countries like Malawi are making strides in tackling VAD, good policy guidance on promotion of OFSP is needed.

REFERENCES

Achir, N. Pénicaud, C. Bechoff, A. Boulanger, R. Dornier, M. & Dhuique-Mayer, C. (2013). Use of Multi-response modelling to investigate mechanisms of β-carotene degradation in dried orange-fleshed sweetpotato during storage: from carotenoids to aroma compounds. Journal of Food Bioprocessing and Technology, 7:1656–1669

Achir, N., Pénicaud, C., Avallone, S., & Bohuon, P. (2011). Insight into β-carotene thermal degradation in oils with multi-response modelling. Journal of the American Oil Chemist's Society, 88(12): 2035– 2045

Bechoff. A & Dhuique-Mayer. C. (2017). Factors influencing micronutrient bioavailability in biofortified crops. Annals of New York Academy of Sciences, 1390: 74–87

Bechoff, A., Dhuique-Mayer, C., Dornier, M., Tomlins, K.I., Boulanger, R., Dufour, D., & Westby, A. (2010). Relationship between the kinetics of beta-carotene degradation and formation of norisoprenoids in the storage of dried sweet potato chips. Journal of Food Chemistry, 121: 348–357

Institute of Medicine. (2001).Dietary Reference Intakes for Vitamin A., Vitamin K., Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, DC.

Tanumihardjo, S. A. (2002). Factors influencing the conversion of carotenoids to retinol:bioavailability to bioconversion to bioefficacy. International Journal for Vitamin and Nutrition Research, 72: 40-45

West, C. E. &. Castenmiller, J. J. J. M. (1998). Quantification of the “SLAMENGHI” factors for carotenoid bioavailability and bioconversion. International Journal of Vitamin and Nutrition Research, 68**:** 371–377

Yeum, K. J., Dos Anjos Ferreira A. L., A., Smith, D. Krinsky, N. I. & Russell, R. M. (2000).The effect of a-tocopherol on the oxidative cleavage of β-carotene. Journal of Free Radical Biology and Medicine, 29 (2,):105–114

Zhang, P., & Omaye, S. T. (2001). Beta-Carotene: interactions with alpha-tocopherol and ascorbic acid in microsomal lipid peroxidation. The Journal of Nutritional Biochemistry, 12(1): 38–45.